Title: Altered kinetics of circulating progenitor cells in cardiopulmonary bypass (CPB) associated vasoplegic patients: An observational study

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Abstract:

Vasoplegia observed post-cardiopulmonary bypass (CPB), is associated with substantial morbidity, multiple organ failure and mortality. Circulating counts of hematopoietic stem cells (HSCs) and endothelial progenitor cells (EPC) are potential markers of neo-vascularization and vascular repair. However, changes in the circulating levels of these progenitors in perioperative CPB and their association with post-CPB vasoplegia remains to be determined. HSC and EPC counts were enumerated at different time-points during CPB in 19 individuals who underwent elective cardiac surgery via flow cytometry. These 19 individuals were categorized into two groups based on severity of post-operative vasoplegia as: clinically insignificant vasoplegic Group 1 (G1) and clinically significant vasoplegic Group 2 (G2). The differential changes in progenitor cell counts during different stages of surgery were compared across these two groups. Machine-learning classifiers (logistic regression and gradient boosting) were employed to determine if differential changes in progenitor counts could differentiate and group subjects based on the severity of vasoplegia. Enumeration of progenitor cells revealed an early and significant increase in the circulating counts of $CD34^+$ and $CD34^+CD133^+$ hematopoietic stem cells (HSC) in G1 subjects which was attenuated in G2 individuals. Additionally, EPCs ($CD34^+VEGFR2^+$) were lower in G2 individuals compared to G1. Gradient boosting outperformed logistic regression in assessing the vasoplegia grouping based on fold change in circulating CD 34^+ levels. Our findings indicate that a lack of early response of $CD34^+$ cells and $CD34^+CD133^+$ HSCs might serve as an early marker for development of clinically significant vasoplegia after CPB.

Keywords: Vasoplegia, Circulating Progenitor cells, Cardiopulmonary Bypass, Machine Learning

1.Introduction:

Vasoplegia is a well recognized post-surgical complication of cardiopulmonary bypass (CPB) [1, 2]. Endothelial activation and dysfunction are considered to be pivotal in the pathophysiology of vasoplegia. Kortekaas *et al* reported that pre-existing endothelial cell activation, determined by increased levels of von Willebrand factor (vWF) and soluble P-selectin at baseline, predisposes patients to vasoplegia [3]. Brettner et al have suggested that deviations in endothelial injury markers in a CPB group as compared to an off-pump surgery group identifies a correlation between ischemia/reperfusion and the extent of endothelial activation [4]. Circulating endothelial cells have also been reported to be elevated at baseline in cardiac surgery patients compared to healthy individuals with their numbers further increasing post CPB [5]. Additionally, impairment in endothelial barrier function is observed during the course of cardiac surgery and post-surgery when assessed through the prism of angiopoetin-Tie2 system [6-8].

Though multiple studies suggest that a compromised endothelium along with continued vascular damage during CPB contributes to the vasoplegic syndrome, the status of vascular repair and vascular progenitors in these settings is ill-characterized. Circulating haematopoietic stem cells (HSCs: $CD34^{+}CD133^{+}$) and endothelial progenitor cells (EPCs: $CD34^{+}VEGFR2^{+}$) are known to home into the site of damaged endothelium and the vessel wall for repair [9]. Reduced numbers and altered function of EPCs are associated with ischemic cardiovascular diseases [10, 11]. However, it is not clear how their circulating counts vary during the unfolding of the vasoplegic syndrome in CPB. We sought to determine the changes in the circulating counts of these progenitors and their relationship to the severity of vasoplegia in cardiac patients undergoing CPB. We also trained machine learning methods (logistic regression and gradient boosting) to assess the accuracy of the vasoplegic categorization of patients based on these markers.

2. Results:

2.1 Characteristics of study subjects:

Drop in the Mean Arterial Pressure (MAP) from pre-CPB to post-CPB despite administration of inotropes and presence of hyperlactemia indicates onset of vasoplegia [1,12]. Hence, we measured intra-arterial systolic blood pressure (SBP), diastolic blood pressure (DBP) and MAP in all 19 study subjects. We observed a significant decrease not only in the MAP post-CPB at 24 hours but also a significant decrease in DBP at 24

hours compared to pre-surgery state (fig. 2A). Additionally, levels of lactate in blood were significantly higher from 1 hour on the pump which peaked at 6 hours and remained significantly high even at 24 hours compared to post-induction (fig. 2B). These parameters were similar for all 19 subjects in our study group, except for difference in dosage of nor adrenaline or nor-epinephrine (NE) infusion post-CPB.

Table 1 shows the baseline anthropometric and clinical characteristics and intake of medications such as Angiotensin II converting enzymes inhibitors (ACEI) and Angiotensin receptor blocker (ARB) of the clinically classified groups (G1 and G2). As shown in table 1, there was no significant difference in age, gender ratio, BMI, heart and respiratory rate, DBP, SBP, and MAP as well as in the % intake of ACEIs and ARBs between the two groups. The percentage of existent comorbidities in recruited subjects such as diabetes, hypertension, dyslipidemia and renal failure is shown in Table 1. A significant increase in urea for G2 is in concordance with observed renal failure in 25% subjects of this group.

The post-operative clinical outcomes for the two groups are shown in table 2. The cardiac function measured as left ventricular ejection fraction (LVEF) post CPB, showed normal values of more than 55% for both the groups (Table 2). Although, MAP was maintained above 70mmHg for both groups with infusion of vasopressors (Table 2), there was significant difference in post CPB characteristics such as duration of ventilation ($p<0.01$), duration of ICU ($p=0.056$) and hospital stay ($p<0.05$) which was higher in case of G2 subjects, confirming severity of clinical vasoplegia in G2 compared to G1 subjects. Among the measured biochemical parameters there was a significant increase in WBC count and a significant decrease in lymphocyte count for G2 compared to G1 subjects (Table 2).

2.2 Circulating levels of progenitor cells

The changes in the circulating levels of progenitors were enumerated in the individuals using flow cytometry. As can be seen in fig. 3A, for the combined 19 on-pump cases, we observed an early increase in $CD34^+$ cells followed by $CD133^+$ and $VEGFR2^+$ cells. $CD34^+$ cells count peaked at 1 hour and remained elevated at 6 hours (25-fold increase compared to post induction), but gradually decreased by 24 hours (fig. 3A). There was a 15-fold increase in $CD133^+$ cell counts, which peaked at 6 hours compared to post induction and then dipped at 24 hours (fig. 3A). In contrast, $VEGFR2^+$ cells had late

response to CPB, which increased gradually post-CPB from 6 hours onwards and remained high at 24 hours (statistically non-significant) (fig. 3A). With regard to double positive cells, EPCs rose from 1 hour onwards and remained elevated at 24 hours (fig. 3B) whereas HSC peaked at 6 hours but dipped by 24 hours (fig. 3B). $CD133^{+}VEGFR2^{+}$ cells did not show any observable change in numbers upon CPB in the study subjects.

2.3 Circulating levels of progenitor cells and levels of lactate in G1 versus G2 subjects

We then sought to determine how the changes in circulating progenitors differs between G1 and G2. As can be seen from figure 4 A and B, the G1 individuals had early increase in the number of $CD34^{+}$ from 0 hour onwards, whereas this increase was delayed in G2 individuals. In G2 individuals, increase in the cell number with regard to $CD34^{+}$ and HSCs was observed only at 6 hours. EPCs started rising from 1 hour onwards in G1 individuals and remained elevated till 24 hours which was blunted in G2 (fig. 4C). The other cell types i.e. $VEGR2^{+}$ cells and $CD133^{+}$ did not show any statistically significant trend for G1 and G2 subjects.

2.4 Assessing vasoplegia grouping for individual patients using machine learning

Log fold change of $CD34^{+}$ marker for each patient was plotted on the y-axis at different time points with respect to three baselines: induction (B1), sternotomy (B2) and 0 hour (P0) against the case number arranged in increasing order of amount of nor-epinephrine dosage given to patients on the x-axis, as shown in figures 5A, 5B and 5C respectively. We observed that on using $CD34^{+}$ as a marker, the 0-hour log fold change with respect to baselines B1 i.e induction (Figure 5A) and B2 i.e sternotomy (Figure 5B) and 24 hours fold change with respect to baseline B2 (Figure 5C) show the clearest visual trend in representing the difference between G1 and G2.

Patients were then classified using logistic regression (Figures 5D, 5E, 5F) and gradient boosting (Figures 5G, 5H, 5I) algorithms on the above-mentioned log fold change values for the $CD34^{+}$ marker. Grouping on each patient were made by leaving that patient out and training on other patients. Gradient boosting outperformed logistic regression in general, with both methods mis-classifying only two patients in the best case (Figures 5D, 5G). The performance of the classifiers was significant ($p<0.05$) for figures 5D, 5E, 5G and 5H (0 hour compared to baselines B1 and B2).

3. Discussion

In the current study, we report differences in time dependent changes in levels of circulating progenitors and EPCs in CPB surgery in individuals exhibiting clinically significant and insignificant vasoplegia. We have demonstrated (as seen in fig. 3A and 3B), $CD34^+$ cells are the first one to respond to CPB followed by $CD133^+$, HSCs, EPCs and $VEGFR2^+$ cells. This part of the study supports conclusions of previous studies investigating the mobilization of progenitor cells in circulation during and after CPB. Our findings on $CD34^+$ and HSCs are comparable with previous studies done by Dotsenko *et al* and Scheubel *et al* [13, 14]. They reported an increase in $CD34^+$ cell counts at the end of surgery which remained elevated 24 hours post-operatively. Similarly, the rise in circulating HSCs, peaks at 6 hours post-operatively and decreases after 24 hours. Increased EPCs and $VEGFR2^+$ levels at the end of the surgery have also been reported by previous studies done by others [15-17]. Notably, the kinetics of progenitor cells and lactate levels follow a similar trend as seen in fig. 3A, 3B and 2B, which suggests hypoxia driven mobilization of progenitor cells [18]. To the best of our knowledge this study for the first time attempts to associate changes in numbers of progenitors with severity of vasoplegia. We have shown that G1 individuals who do not develop clinically significant vasoplegia after surgery showed significant early increase in the number of $CD34^+$ and HSCs. This rise was not observed in the individuals who developed significant vasoplegia (G2). Similarly, blunted response of EPCs was observed in G2 individuals compared to G1.

Upon categorization based on severity of vasoplegia, the early response of $CD34^+$ and HSC counts in G1 as seen in fig. 4A and 4B might indicate the body's attempt to limit vascular damage by inducing repair mechanism against the insult caused due to CPB. This early response is compromised in the case of G2 individuals. Prior studies have claimed that $CD34^+$ cells through paracrine signaling promote therapeutic angiogenesis in response to myocardial ischemia and has the potential to ensure engraftment of transplanted cells [19]. Recently, Cogle *et al* have shown that increase in the number of $CD34^+$ cells in bone marrow is directly associated with improved functional outcome after acute myocardial infarction (AMI) in the Timing in Myocardial Infarction Evaluation (TIME) and Late TIME clinical trials [20]. In another study performed by Fadini *et al* have found that $CD34^+$ cells have a strong negative correlation with cardiovascular risk [21]. Similarly, HSCs are known to promote neovascularization on

tissue engineered construct and have shown significant correlation with autologous white cell count and engraftment kinetics in myeloma patients [22, 23]. Based on these studies and our observations, we speculate that endothelial injury caused by CPB if not accompanied by an early mobilization of sufficient circulating progenitor cells may result in impaired vascular repair thereby contributing to the severity of vasoplegia. However, the reason for delayed response of $CD34^+$ and HSCs cells in G2 individuals are still unknown. Given the variability of vasoplegia even among patients within different risk groups it would be reasonable to assume that pre-operative profiles may influence the ability to mobilize circulating progenitors and HSCs.

Furthermore, we observed a depression in EPCs count in G2 patients as seen in fig.4C. The reason for decreased circulating counts of EPCs in G2 could be due to compromised migration, homing and reparative potential of these cells as shown by Ruel *et al*. They demonstrated that the migratory effect of EPC was compromised in on-pump CPB patients [17]. Further, Lei Du *et al* have reported that EPCs start homing to the injured site after 4 hours from the end of the surgery and reported a drop in the counts of circulating EPCs after 4 hours with a corresponding increase in their numbers in lung and kidney tissues [24].

To support our findings, we employed machine learning techniques to evaluate our categorization of groups G1 and G2 with respect to the $CD34^+$ marker. We found that the log fold change values of $CD34^+$ markers (as seen in fig.5A, 5B and 5C) reflected our findings in terms of an increasing trend in G1 and decreasing trend in G2. Among the algorithms we utilized, namely logistic regression and gradient boosting, we found that gradient boosting gave a superior performance in classifying patients into G1 and G2 as seen in fig 5G, 5H, 5I. We found that in most patients, clinical classification agreed with the grouping from gradient boosting. However certain patients namely patients 6, 8 and 9 were frequently misclassified. Of these, patient 9 had Pre-CPB renal failure, while patients 6 and 8 had borderline levels of vasoplegia as indicated by norepinephrine dosage. While these results are promising, a study on a larger cohort would enable us to refine our predictive methods for better understanding.

4. Methods:

4.1 Materials:

Vacutainers for blood collection K2-EDTA (Cat: 367863) and serum separation (Cat: 367812), were purchased from Becton Dickinson Biosciences. Fluorescent antibodies such as anti-human CD133-Phycoerythrin (PE) (Cat: 130-080-801) was from Miltenyi Biotech, anti-human CD34-Fluorescein isothiocyanate (FITC) (Cat: 343604), anti-human VEGFR2-Allophycocyanin (APC) (Cat: 359916) and isotype control antibodies like mouse IgG2a-FITC (Cat: 400207) and mouse IgG1k-PE (Cat: 400113), were purchased from BioLegend. Other dry chemicals were purchased from Sigma-Aldrich.

4.2 Study design and sample collection:

Following informed consent, subject recruitment and sample collection were done at the Cardiothoracic Surgical unit of Apollo Hospitals, Chennai. The study design was reviewed and approved by the Institute Ethics Committees of Apollo Hospital [AMH-001/01-18] as well as the Indian Institute of Technology Madras [IEC# IEC/2016/02/MDX-2/05]. A total of 19 elective cardiac surgery individuals (8 male and 11 females), age range 40-70 years, undergoing CPB were recruited. Individuals with emergency trauma surgery or any infectious diseases were excluded.

The blood samples were collected in K_2-EDTA vacutainers for subsequent flow cytometry from central venous catheter placed in the jugular vein when the patient was not on the CPB pump. When the patient was on pump, blood was collected from the inlet of the pump at specified time intervals as explained below. The whole blood collected was processed within 2 hours of collection for flow cytometry. The time points for blood collection were: (1) 'Post-Induction' (time after the induction of anaesthesia prior to the start of surgery); (2) 'Sternotomy' (beginning of surgery after median sternotomy); (3) '0 hour' (start of the CPB pump); (4) '1 hour' (1 hour on pump); (5) '6 hour'(end of surgery when the patient was shifted to ICU) (6) '24 hour' (time point when the patient is in ICU for recovery). These time points and study design are summarized in figure 1. In order to minimize inter patient bias which may contribute to variability, we have considered each patient's baseline i.e. 'Post-Induction' as respective control.

4.3 Grouping of subjects based on severity of vasoplegia:

In the absence of a standard consensus for the definition of clinical vasoplegia [25, 26], we choose to categorize our recruited subjects based on total dose of nor epinephrine (NE) infused in these subjects. In most cardiothoracic units low levels of NE (usually total dose <0.02mg/kg body weight) is given in all patients post-surgery. This dosage is increased only if a patient further develops vasoplegia. We thus reasoned that increased use of pressor beyond 0.02mg/Kg body weight identifies clinical worsening of vasoplageia. Hence, we choose a cutoff dose of total NE above 0.02mg/kg body weight to group the subjects into two groups. Among the recruited 19 subjects, 11 subjects received NE <0.02mg/Kg body weight and were classified as a clinically insignificant vasoplegia group or group 1 (G1). Remaining 8 subjects which received NE >0.02mg/Kg body weight were termed as clinically significant vasoplegia group or group 2 (G2).

4.4 Enumeration of circulating progenitor cells via flow cytometry:

For enumeration of circulating vascular progenitors in whole blood, cells were fixed and erythrocytes were lysed using fluorescence-activated cell sorter (FACS) lysing solution. Blocking was performed with 5% Fetal Bovine Serum (FBS) and 0.2 % Bovine Serum Albumin (BSA) for 30 min followed by staining with CD133-PE, CD34-FITC and VEGFR2-APC antibodies for 40 minutes at 4°C. Corresponding isotype immunoglobulin G (IgG) 1-PE and IgG2a-FITC and IgG2aκ-APC antibodies were used as controls. A minimum of 5 lakhs events were acquired and scored as per EUROSTAR guidelines using FACS Aria flow cytometer (Becton Dickinson) [27]. Data were analyzed using the Flowjo software program (Version 7.6.1, Tree Star Inc.). Following appropriate gating, CD34^{+}, CD133^{+}, VEGFR2^{+}, EPC (CD34^{+}VEGFR2^{+}), HSC (CD34^{+}CD133^{+}) and CD133^{+}VEGFR2^{+} cells (Supplementary figure) were enumerated from lympho-monocyte fraction and are represented as the number of cells per million lympho-monocytic events.

4.5 Statistical analysis of clinical data:

Progenitor cell counts are expressed as mean ± SEM of the number of cells counted per 1 million lymphomonocyte events. Clinical data are also expressed as mean ± SEM.

Normal distribution of the data was confirmed through Kolmogorov-Smirnov test. Comparisons within and across groups were performed by paired and unpaired Student's t-test, respectively. For non-parametric data, Mann-Whitney's test and Wilcoxon matched pair test was employed.

4.6 Mathematical model to assess the characterization of study subjects

We used two machine learning techniques, logistic regression and gradient boosting, as prediction tools. Logistic regression is a machine learning tool used for binary classification problems. In linear regression, a linear function of inputs is fitted to match output on training data. The output is a real number. In logistic regression, the output value is transformed using a cost function known as a logistic or sigmoid function to return a value between zero and one, which can be interpreted as a probability value. This output can be thresholder to predict a value of 0 or 1. We used the implementation in scikit-learn [28].

Gradient boosting is a decision-tree-based algorithm for classification problems [29]. A decision tree is a tree of possible choices to be made given the input data. In a classification and regression tree (CART), unlike a traditional decision tree, the leaves indicate possible scores for outcomes, rather than binary choices. In gradient boosting, an ensemble of trees is used where each tree is trained to minimize errors of preceding trees. This has become a very popular machine-learning tool for tabular data in recent years, here we use the XGBoost package [30].

Based on input parameters, logistic regression and gradient boosting were used to predict whether a patient belongs to G1 or G2. Predictions were validated using "leave one out"-- each patient was left out once, and the machine-learning model was trained on the remaining patients and tested on the left-out patient. The significance of these predictions was assessed using *p*-values, i.e. the probability of a similar or better fit happening by chance. To calculate these, we compared the results of 10,000 randomizations. Random data were generated by shuffling the vasoplegia categorization (that is, redistributing the category labels among the patients, ensuring that there are still the same number of patients as are there in G1 and G2. Here, the *p*-value is the fraction of random data-sets with a similar or lower mean absolute error (MAE; the average of the absolute value of the error over all individuals) to the real data. Similar results (not shown) were obtained with a more aggressive randomization, where for each patient, we

retain the vasoplegia categorization but pick a random number between the minimum and maximum of the original graph as the log fold-change value.

5. Study limitations

There are certain limitations of the current study. For instance, due to small sample size, certain observations did not reach statistical significance. The other major limitation of the study is non-availability of the blood sample beyond 24 hours which has confined us to observe the kinetics of progenitor cells up to 24 hours. Additionally, we did not measure the levels of secretory endothelial markers during perioperative time points to assess endothelial damage. Further studies with large cohorts tracking the changes both between and within risk stratified groups undergoing cardiopulmonary bypass will be needed to determine if a particular response profile could be used to predict the occurrence of significant vasoplegia.

Reference List

1. Levin,M.A. et al. Early on-cardiopulmonary bypass hypotension and other factors associated with vasoplegic syndrome, Circulation 120, 1664-1671 (2009).

2. Omar,S., Zedan,A., & Nugent,K. Cardiac vasoplegia syndrome: pathophysiology, risk factors and treatment, Am. J. Med. Sci. 349, 80-88 (2015).

3. Kortekaas,K.A. et al. Pre-existing endothelial cell activation predicts vasoplegia after mitral valve surgery, Interact. Cardiovasc. Thorac. Surg. 17, 523-530 (2013).

4. Brettner,F. et al. Vascular Endothelial Dysfunction during Cardiac Surgery: On Pump versus Off-Pump Coronary Surgery, Eur. Surg. Res. 58, 354-368 (2017).

5. Schmid,F.X. et al. Direct evidence of endothelial injury during cardiopulmonary bypass by demonstration of circulating endothelial cells, Perfusion 21, 133-137 (2006).

6. Hilbert,T. et al. Endothelial permeability following coronary artery bypass grafting: an observational study on the possible role of angiopoietin imbalance, Crit Care 20, 51 (2016).

7. Jongman,R.M. et al. Angiopoietin/Tie2 Dysbalance Is Associated with Acute Kidney Injury after Cardiac Surgery Assisted by Cardiopulmonary Bypass, PLoS. One. 10, e0136205 (2015).

8. Koning,N.J. et al. Endothelial hyperpermeability after cardiac surgery with cardiopulmonary bypass as assessed using an in vitro bioassay for endothelial barrier function, Br. J. Anaesth. 116, 223-232 (2016).

9. Urbich,C. & Dimmeler, S. Endothelial progenitor cells: characterization and role in vascular biology, Circ. Res. 95, 343-353 (2004).

10. Grisar,J.C., Haddad,F., Gomari,F.A., & Wu,J.C. Endothelial progenitor cells in cardiovascular disease and chronic inflammation: from biomarker to therapeutic agent, Biomark. Med. 5, 731-744 (2011).

11. Schmidt-Lucke,C. et al. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair, Circulation 111, 2981-2987 (2005).

12. Welbourn,N., Melrose,D.G., & Moss,D.W. Changes in serum enzyme levels accompanying cardiac surgery with extracorporeal circulation, J. Clin. Pathol. 19, 220-232 (1966).

13. Dotsenko,O. et al. Bone marrow resident and circulating progenitor cells in patients undergoing cardiac surgery. Ann.Thorac.Surg 90, 1944-1951 (2010).

14. Scheubel,R.J. et al. Age-dependent depression in circulating endothelial progenitor cells in patients undergoing coronary artery bypass grafting, J. Am. Coll. Cardiol. 42, 2073-2080 (2003).

15. Roberts,N., Xiao,Q., Weir,G., Xu,Q., & Jahangiri,M. Endothelial progenitor cells are mobilized after cardiac surgery, Ann. Thorac. Surg. 83, 598-605 (2007).

16. Gill,M. et al. Vascular trauma induces rapid but transient mobilization of VEGFR2(+)AC133(+) endothelial precursor cells, Circ. Res. 88, 167-174 (2001).

17. Ruel,M. et al. Effects of off-pump versus on-pump coronary artery bypass grafting on function and viability of circulating endothelial progenitor cells, J. Thorac. Cardiovasc. Surg 130, 633-639 (2005).

18. Schroder,K. et al. NADPH oxidase Nox2 is required for hypoxia-induced mobilization of endothelial progenitor cells, Circ. Res. 105, 537-544 (2009).

19. Mackie,A.R. & Losordo,D.W. CD34-positive stem cells: in the treatment of heart and vascular disease in human beings, Tex. Heart Inst. J. 38, 474-485 (2011).

20. Cogle,C.R. et al. Detailed analysis of bone marrow from patients with ischemic heart disease and left ventricular dysfunction: BM CD34, CD11b, and clonogenic capacity as biomarkers for clinical outcomes, Circ. Res. 115, 867-874 (2014).

21. Fadini,G.P. et al. Circulating CD34+ cells, metabolic syndrome, and cardiovascular risk, Eur. Heart J. 27, 2247-2255 (2006).

22. Herrmann,M. et al. CD34/CD133 enriched bone marrow progenitor cells promote neovascularization of tissue engineered constructs in vivo, Stem Cell Res. 13, 465-477 (2014).

23. Hicks,C., Wong,R., Manoharan,A., & Kwan,Y.L. Viable CD34+/CD133+ blood progenitor cell dose as a predictor of haematopoietic engraftment in multiple myeloma patients undergoing autologous peripheral blood stem cell transplantation, Ann. Hematol. 86, 591-598 (2007).

24. Du,L *et al*. Endothelial Progenitor Cell Homing Decreases Postoperative Complications in Patients Undergoing Cardiac Surgery with Cardiopulmonary Bypass. Journal of Anesthesia and Perioperative Medicine, Journal of Anesthesia and Perioperative Medicine , 15-24 (2014).

25. Chan,J.L. et al. Characterizing Predictors and Severity of Vasoplegia Syndrome After Heart Transplantation. Ann. Thorac. Surg. 105, 770-777 (2018).

26. Lambden,S., Creagh-Brown,B.C., Hunt,J., Summers,C., & Forni,L.G. Definitions and pathophysiology of vasoplegic shock, Crit Care 22, 174 (2018).

27. Distler,J.H. et al. EULAR Scleroderma Trials and Research group statement and recommendations on endothelial precursor cells, Ann. Rheum. Dis. 68, 163-168 (2009).

28. L.Buitinck. API design for machine learning software: experiences from the scikit-learn project, Proceedings of ECML PKDD Workshop: Languages for Data Mining and Machine Learning , 108-122 (2013).

29. Friedman, J.H. Greedy function approximation: a gradient boosting machine. Annals of statistics , 1189-1232 (2001).

30. Chen,T. XGBoost: A Scalable Tree Boosting System, Proceedings of the 22nd ACM SIGKDD International Conference on Knowledge Discovery and Data Mining , 785-794 (2016).

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Author Contributions

SN contributed in sample collection, processing of sample for flow cytometry. She carried out the analysis and interpretation of flow cytometry data, did the statistical analysis and wrote the manuscript. CK contributed in computational analysis and machine learning prediction. URP contributed in sample collection, clinical data compilation and interpretation. AAN performed statistical analysis and drafted the manuscript. RS contributed in computational analysis and machine learning prediction. GIM contributed in computational analysis and machine learning prediction. JG revised the manuscript critically for important intellectual content. MD made contributions in conception, designing of the experimental study, supervised flow cytometry data and in the revision of the manuscript critically for important intellectual content. PRT made contributions in conception, designing of the study, finding clinical correlation and interpretation of the data and in the revision of the manuscript critically for important intellectual content.

Conflict of Interest Statement: No conflict of interest

FIGURE LEGENDS

Figure 1: Study design showing time points of sample collection.

Figure 2: Characterization of study subjects. (A) Graph showing blood pressures changes between Pre-Operation and Post-Operation in all the study individuals (n=19). (B) Lactate concentration from blood at different time points in all the individuals (n=19) measured in mmol/L. Data shown as Mean ± SEM. ** $p \leq 0.01$, ***$p \leq 0.001$, **** $p \leq 0.0001$ vs respective control. Mann-Whitney's test and Wilcoxon matched pair test were employed accordingly.

Figure 3: Fold changes in circulating counts of measured progenitor cells for all 15 on pump cases (4 were considered outlier by Statistical Package for the Social Sciences). (A) Line graph showing fold change in single positive cell counts for $CD34^+$, $CD133^+$& $VEGFR2^+$ cells and (B) Line graph summarizing changes in double positive cell counts for HSCs ($CD34^+CD133^+$) and EPCs ($CD34^+VEGFR2^+$). Data is plotted as mean ± SEM. *$p \leq 0.05$, ** $p \leq 0.01$ vs post induction, $ $p \leq 0.05$, $$ $p \leq 0.01$ vs sternotomy and # vs 6 hours. Mann-Whitney's test and Wilcoxon matched pair test were employed accordingly.

Figure 4: Circulating counts of progenitor cells in individuals undergoing CPB at various intra-operative time points in G1 and G2 study individuals. Line graph showing fold change in cell counts for (A) $CD34^+$ (B) HSC ($CD34^+CD133^+$) and (C) EPC ($CD34^+VEGFR2^+$) in vasoplegic groups namely G1 and G2. Data is plotted as mean ± SEM. *$p \leq 0.05$, ** $p \leq 0.01$ vs G2 at that point. Mann-Whitney's test and Wilcoxon matched pair test were employed accordingly.

Figure 5: Bar graph showing log fold change of $CD34^+$ marker for each patient, identified by case number, computed at different time points with respect to three baselines: induction, sternotomy and 0 hour. (A) Log fold change at on-pump 0hr with respect to induction. (B) Log fold change for on-pump 0hr time point with respect to sternotomy. (C) Shows 24hr log fold change with respect to sternotomy. Case numbers for patients in G1 (insignificant vasoplegia) are shown in green and those for patients in G2 (significant vasoplegia) are shown in red. The status of individual patients as diabetic (d), hypertensive (h) or as having renal failure (r) is also indicated together with each bar. (D),(E),(F) show our machine learning groupings for G1 and G2 using logistic regression and (G),(H),(I) show the same based on gradient boosting. Correctly

classified patients are shown, by case number, in green boxes, and misclassified patients in red boxes. Corresponding p-values are shown.

Table 1: Anthropometric and clinical characteristics of CPB individuals at baseline.

Parameters	Group 1 (n=11)	Group 2 (n=8)
Age (yrs)	57 ± 2	54 ± 2
Sex (M / F)	5/6	3/5
BMI	25.51± 1.02	26.46 ± 2.38
Heart Rate (per min)	83.45 ± 3.32	89 ± 6.27
Systolic BP (mmHg)	121.8 ± 2.26	115.0 ± 4.62
Diastolic BP (mmHg)	78.18 ± 2.26	72.5 ± 2.50
Mean Arterial Pressure (mmHg)	92.59 ± 2.53	86.67 ± 2.88
Respiratory Rate (No. of breaths/min)	20.18 ± 0.68	22.13 ± 1.23
Urea (mg/dl)	24.80 ± 2.19	45.29 ± 6.7**
Creatinine (mg/dl)	0.74 ± 0.05	0.94 ± 0.12
Comorbidities % prevalence		
Diabetes (%)	45.0	37.0
Hypertension (%)	45.0	25.0
Dyslipidemia (%)	9.0	12.0
Renal failure (%)	0	25.0
Medication intake (% per group)		
ACEI (%)	27.27	35.1
ARB (%)	27.27	12.5

*Intake of Angiotensin II converting enzymes inhibitors (ACEI) and Angiotensin receptor blocker (ARB) are expressed in percentage, Data expressed in Mean± S.E.M. **p<0.01*

Table 2: Clinical and Biochemical characteristics of CPB individuals at post CPB

Post-CPB (at ICU) measurements			
Clinical parameters		Group 1 (n=11)	Group 2 (n=08)
Heart Rate (per min) at 24 hrs		77.82 ± 4.14	95.00 ± 5.57 *
Systolic BP (mmHg) at 24 hrs		124.1 ± 4.50	102.3 ± 4.74 **
Diastolic BP (mmHg) at 24 hrs		59.55 ± 2.00	55.63 ± 1.36
Mean Arterial Pressure (mmHg) at 24 hrs		77.64 ± 1.82	71.63 ± 1.96 *
Respiratory rate (No. of breaths/min)		20.36 ± 0.88	22.00 ± 1.30
LVEF (%)		59.63 ± 1.78	57.86 ± 2.14
Duration of ventilation (hrs)	CMV	12.30 ± 1.10	18.13 ± 3.07
	CPAP	2.37 ± 0.46	22.25 ± 16.59 **
ICU Stay (days)		4.50 ± 0.61	10.63 ± 4.46
Hospital stay (days)		9.81 ± 0.90	23.25 ± 5.9*
Death Rate (%)		0.0	25.0
Biochemical measurements			
Urea (mg/dl)		33.27 ± 5.34	36.86 ± 3.77
Creatinine (mg/dl)		0.73 ± 0.08	0.96 ± 0.09*
EGFR (ml/min/1.73m2)		116.9 ± 7.41	93.32 ± 12.74
Hemoglobin (%)		10.03 ± 0.40	10.33 ± 0.40
ESR (mm/hr)		53.60 ± 7.58	40.00 ± 9.33
WBC (103/mm3)		14.02 ± 2.06	20.62 ± 2.82 *
Lymphocytes (%)		10.55 ± 2.08	5.12 ± 0.85 *
Monocytes (%)		7.09 ± 1.03	7.12 ± 1.00
Neutrophils (%)		80.18 ± 3.46	86.88 ± 1.69
Platelet count (103/mm3)		187.2 ± 11.23	192.6 ± 39.17

*EGFR - Estimated Glomerular Filteration Rate; ESR - Erythrocyte Sedimentation Rate; WBC - White Blood Cells; CMV - Continuous mandatory ventilation; CPAP - Continuous positive airway pressure; ACEI - Angiotensin Converting Enzymes inhibitor; ARB - Angiotensin II receptor blocker;LVEF - Left ventricular ejection fraction; Data expressed in Mean ± S.E.M. LVEF, Hemoglobin and Death Rate are expressed in %. *p<0.05 and **p<0.01.*

FIGURE 1

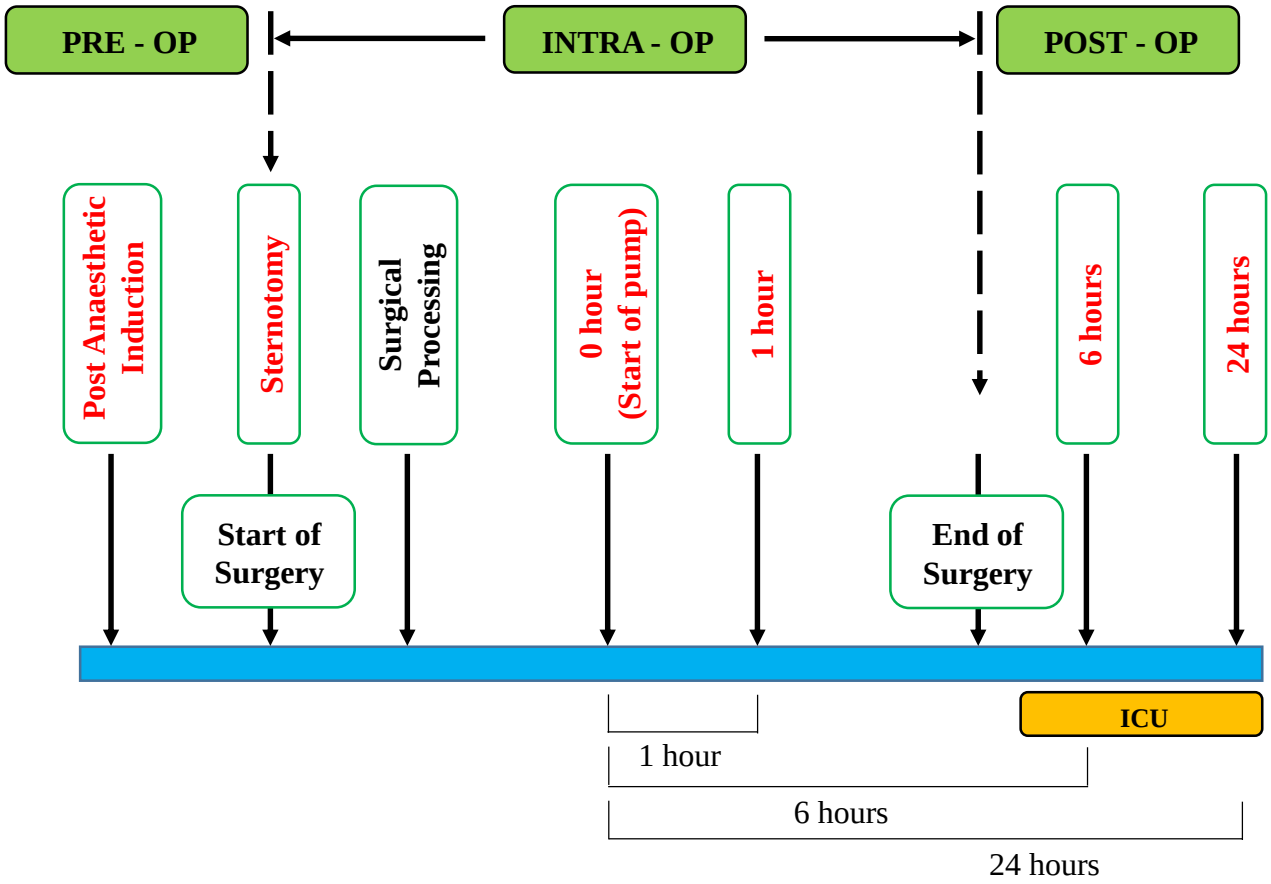

PRE - OP
INTRA - OP
POST - OP
Post Anaesthetic Induction
Sternotomy
Surgical Processing
0 hour (Start of pump)
1 hour
6 hours
24 hours
Start of Surgery
End of Surgery
ICU
1 hour
6 hours
24 hours

FIGURE 2

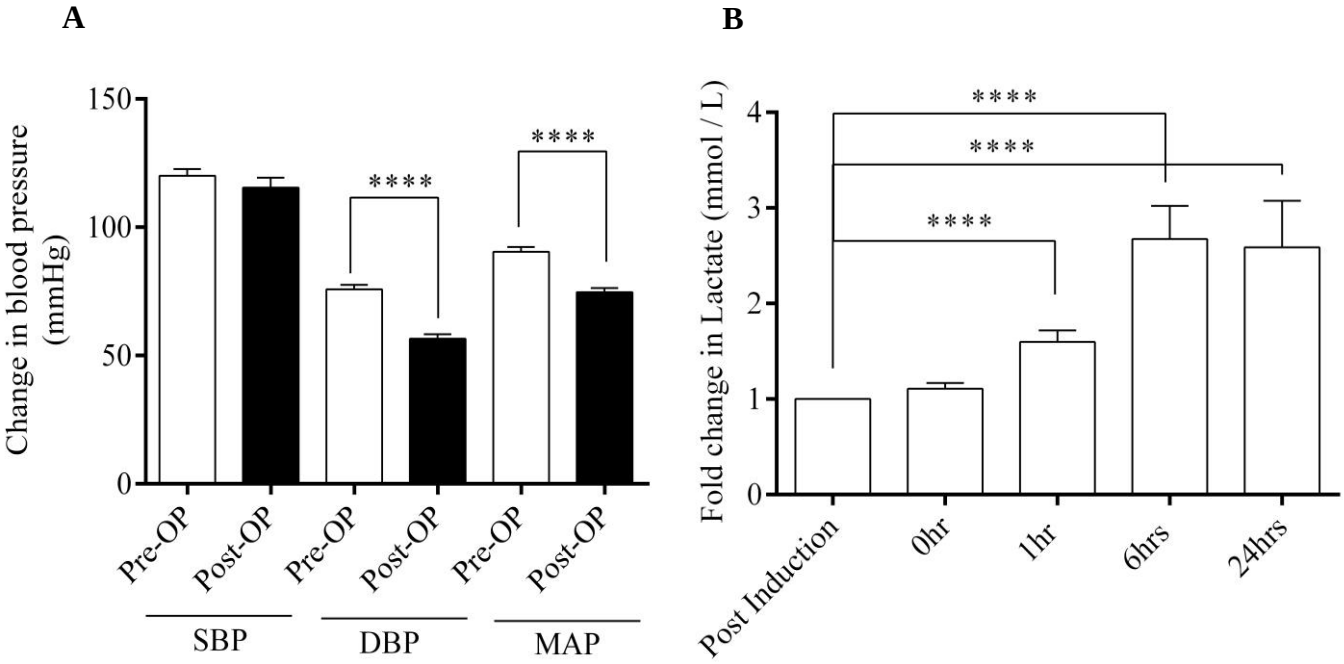
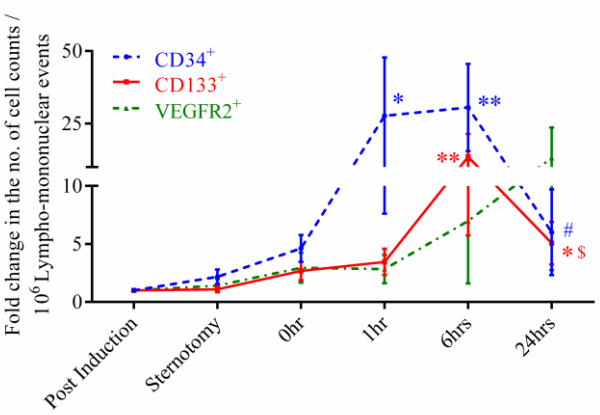

A
B
Change in blood pressure (mmHg)
150
100
50
0
****
****
Pre-OP
Post-OP
Pre-OP
Post-OP
Pre-OP
Post-OP
SBP
DBP
MAP
Fold change in Lactate (mmol / L)
4
3
2
1
0
****
****
****
Post Induction
0hr
1hr
6hrs
24hrs

FIGURE 3

A


Fold change in the no. of cell counts / 10^6 Lympho-mononuclear events
CD34+
CD133+
VEGFR2+
50
25
10
5
0
*
**
**
#
* $
Post Induction
Sternotomy
0hr
1hr
6hrs
24hrs

B

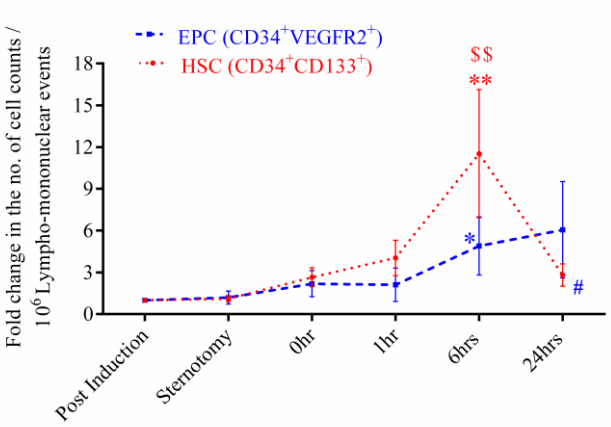

Fold change in the no. of cell counts / 10^6 Lympho-mononuclear events
EPC (CD34+VEGFR2+)
HSC (CD34+CD133+)
18
15
12
9
6
3
0
$$
**
*
#
Post Induction
Sternotomy
0hr
1hr
6hrs
24hrs

FIGURE 4

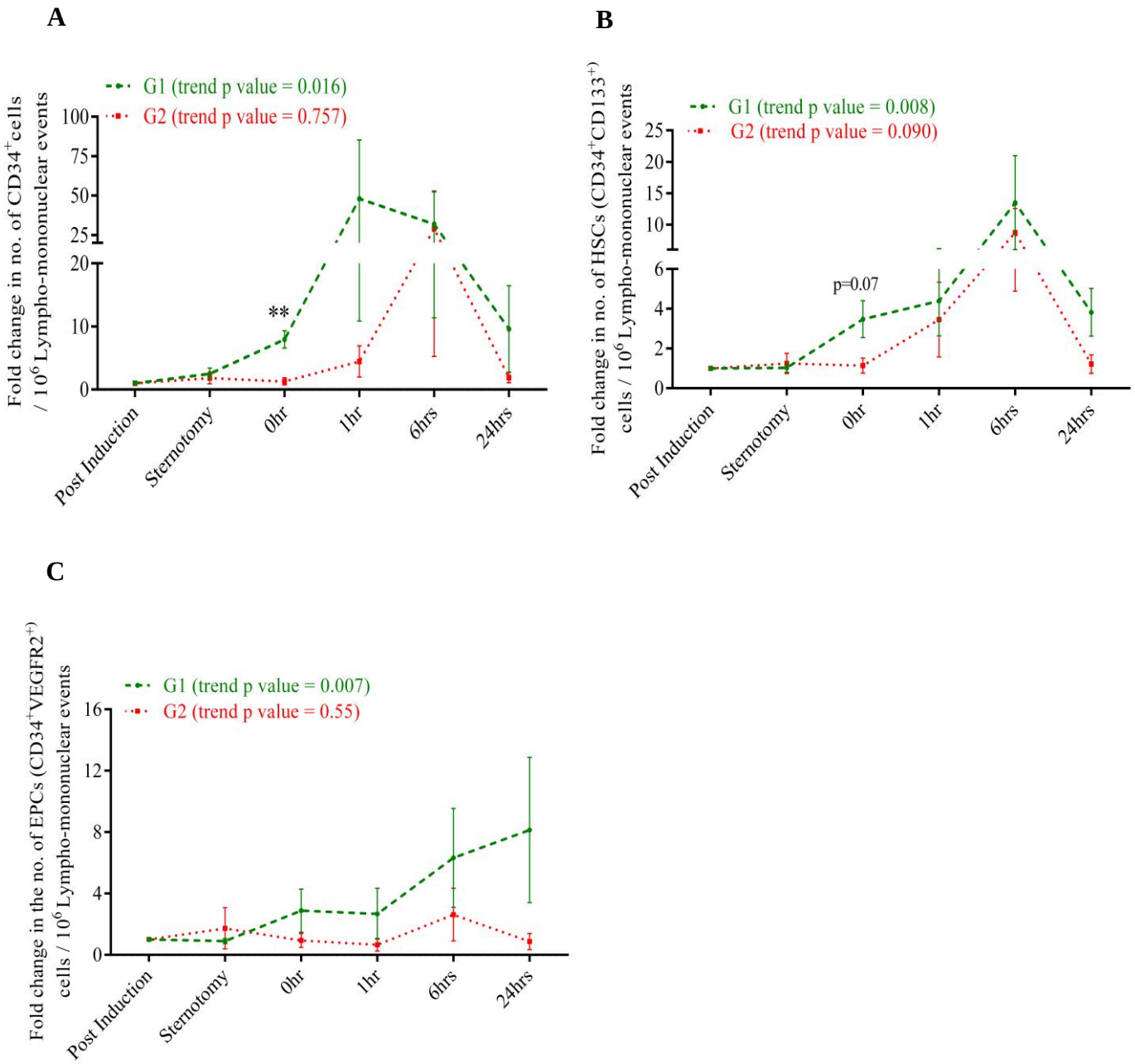
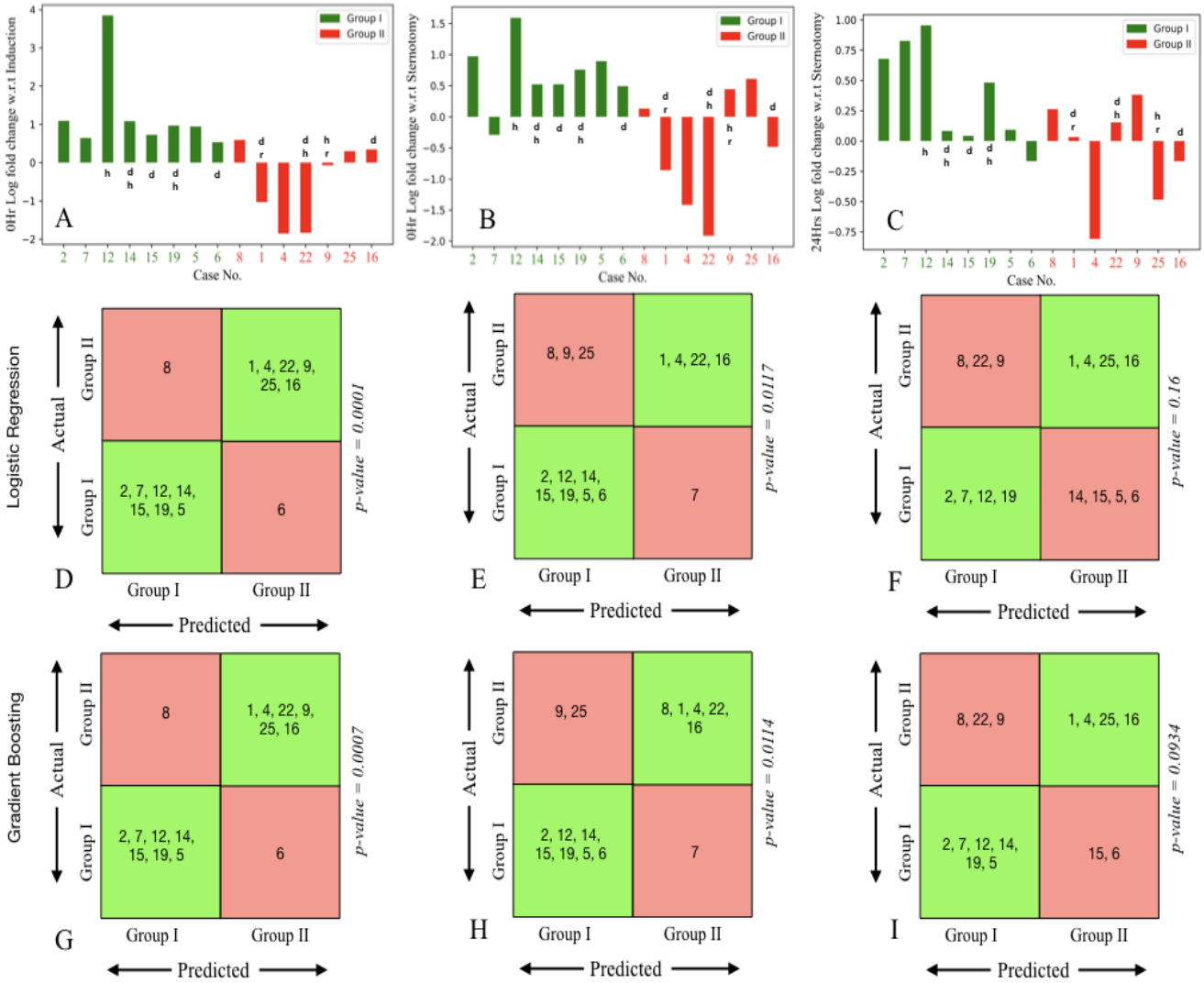

A
G1 (trend p value = 0.016)
G2 (trend p value = 0.757)
Fold change in no. of CD34+cells / 10^6 Lympho-mononuclear events
100
75
50
25
20
10
0
**
Post Induction
Sternotomy
0hr
1hr
6hrs
24hrs
B
G1 (trend p value = 0.008)
G2 (trend p value = 0.090)
Fold change in no. of HSCs (CD34+CD133+) cells / 10^6 Lympho-mononuclear events
25
20
15
10
6
4
2
0
p=0.07
Post Induction
Sternotomy
0hr
1hr
6hrs
24hrs
C
G1 (trend p value = 0.007)
G2 (trend p value = 0.55)
Fold change in the no. of EPCs (CD34+VEGFR2+) cells / 10^6 Lympho-mononuclear events
16
12
8
4
0
Post Induction
Sternotomy
0hr
1hr
6hrs
24hrs

FIGURE 5


A
0Hr Log fold change w.r.t Induction
Case No.
B
0Hr Log fold change w.r.t Sternotomy
Case No.
C
24Hrs Log fold change w.r.t Sternotomy
Case No.
Group I
Group II
Logistic Regression
Gradient Boosting
Actual
Predicted
D
8
1, 4, 22, 9, 25, 16
2, 7, 12, 14, 15, 19, 5
6
p-value = 0.0001
E
8, 9, 25
1, 4, 22, 16
2, 12, 14, 15, 19, 5, 6
7
p-value = 0.0117
F
8, 22, 9
1, 4, 25, 16
2, 7, 12, 19
14, 15, 5, 6
p-value = 0.16
G
8
1, 4, 22, 9, 25, 16
2, 7, 12, 14, 15, 19, 5
6
p-value = 0.0007
H
9, 25
8, 1, 4, 22, 16
2, 12, 14, 15, 19, 5, 6
7
p-value = 0.0114
I
8, 22, 9
1, 4, 25, 16
2, 7, 12, 14, 19, 5
15, 6
p-value = 0.0934