Statistical Analysis of Blood Cells and Blood Proteins Behaviours contacted with PMEA-coated and Uncoated Oxygenators

PMEA-kaplı ve Kapsız Oksijenatörlerle Temas Eden Kan Hücrelerinin ve Kan Proteinlerinin Davranışının İstatistiksel Analizi

Research Article

Fatma Ayhan[1], Mustafa Kocakulak[2], Oğuz Akpolat[1], Hakan Ayhan[1,*]

[1]Muğla University, Faculty of Science, Department of Chemistry, Muğla, Turkey.

[2]Başkent University, Faculty of Engenering, Biomedical Engineering Department, Ankara, Turkey.

ABSTRACT

The blood compatibility of the polymethoxyethyle acrylate (PMEA) coated and uncoated hollow fibers were investigated using multivariate statistical methods. The patients were divided into two groups where one is PMEA-coated fibers used patients and the other is uncoated oxygenator fibers used patients. The two groups were evaluated by total bilirubin, direct bilirubin, erythrocyte, albumin, fibrinogen, leukocyte, and total protein analysis results. Three evaluations were taken for comparison: baseline (T1), after protamine injection (T4) and intensive care (T5). There are no significant differences in the comparison. Additionally, the measurements were also compared by age. The patients were divided to four different age groups and no differences were found between the age groups except intensive care fibrinogen value and baseline protrombine time (Pt) Value. Then attempt were made to look for the significant correlation value of the measurements. After significant one was found, one measurement value was taken as dependent variable and the measurements, which are highly correlated with dependent variable as independent variables in the regression model. All of the models established that the analysis were significant with both squared r, p and F values. In some regression models, only interaction effect was seen with the variables, which means that PMEA-coating created more hydrophilic surfaces for protein adsorption when compared with uncoated Cardiopulmonary by pass filters.

Key Words:

PMEA-coated and uncoated oxygenator, by-pass, age groups, correlations, regression, comparison, multivariate statistics method

ÖZET

Polimetoksietilen akrilat (PMEA) kaplı ve kapsız oyuk fiberlerin kan uyumluluğu multivariat istatistiksel yöntemler kullanılarak incelenmiştir. Hastalar iki gruba ayrılmıştır: biri PMEA kaplı diğeri de kapsız oksijeneratör fiberlerleri kullanan hastalar. Her iki grup da toplam bilirubin, direkt bilirubin, eritrosit, albumin, fibrinojen, lökosit ve toplam protein analiz sonuçları ile değerlendirilmiştir. Karşılaştırma için üç düzey alınmıştır: taban seviyesi (T1), protamin enjeksiyonu sonrası (T4) ve yoğun bakım (T5). Düzeyler arasında belirgin farklılıklar gözlenmemiştir. Ek olarak ölçümler yaşa göre de karşılaştırılmıştır. Hastalar dört farklı yaş grubuna ayrılmıştır ve yoğun bakım fibrinojen değeri ve taban seviyesi protrombin zamanı (Pt) değeri hariç yaş grupları arasında bir fark bulunmamıştır. Sonra ölçümlerin önemli ilişki değerini bulmak için deneme yapıldı. Önemli bir tane bulunduktan sonra regrasyon modelinde, bir ölçüm bağımlı değişken olarak ve bağımlı değişkenle çok iyi bağlantı kuran ölçümler de bağımsız değişkenler olarak alınmıştır. Analizde kurulan modellerin hepsinin r^2, p ve F değerleri önemlidir. Bazı regrasyon modellerinde sadece etkileşim etkisi değişkenlerle birlikte görülmüştür ki bunun anlamı, Kaplanmış cardiopulmoner by pass filtreleri, kaplanmamışlar ile karşılaştırıldığında PMEA kaplılar protein adsorpsiyonu için daha hidrofilik yüzeyler oluşturmuştur.

Anahtar Kelimeler:

PMEA-Kaplı ve kapsız oksijenatör, by-pas, yaş grupları, Korelasyon, Bağlanım Kıyaslama, Çok değişkenli İstatistik yöntemi.

Article History: Received July 11, 2012; Revised July 28, 2012; Accepted August 03, 2012; Avaliable Online: August 17, 2012

Correspondence to: Hakan Ayhan, Mugla University, Faculty of Science, Department of Chemistry, Biochemistry Division, 48000 Muğla, Turkey

Tel: +90 252 211 1506 Fax: +90 252 211 1472 E-Mail: hayhan48@gmail.com

Abbreviations
CPB: Cardio Pulmonary Bypass
PMEA: PolyMethoxyEthyleacrylate;
tbil.: total bilirubin.
dbil.: direct bilirubin.
erit.: erythrocyte.
Alb.: albumin.
fibn.: fibrinogen.
leuk.: leukocyte.
tprot.: total protein.
T1: baseline
T2: In CPB
T3: End of CPB
T4: after protamine injection
T5: intensive care.
Aptt: Activated Partial Tromboplastine Time
Pt:Prothrombine time
IVs: independent variables
DVs: dependent variables
Sign.: significant

INTRODUCTION

Multivariate statistics are increasingly popular techniques used for analyzing complicated data sets. They provide analysis when there are many independent variables (IVs) and/or many dependent variables (DVs), all correlated with one another to varying degrees. Multivariate methods are more complex than univariate by at least an order of magnitude. One answer to the question "Why multivariate statistics?" is that the techniques are now accessible by computer. Only the most dedicated number cruncher would consider doing real-life-sized problems in multivariate statistics without a computer. Fortunately, excellent multivariate programs are available in a number of computer packages. Three packages are most used in computers: SPSS (Statistical Package for the Social Sciences), SAS (SAS Institute Inc.) and SYSTAT (SPSS Inc.). The statistical computer programs increase the degree of correctness, quickness and simplicity of the analyses. Recent versions of the programs are implemented in windows, with menus that permit at least some of the techniques illustrated in this study to be analyzed. All of the techniques may be implemented through syntax, and some of the syntax itself may be generated through menus.

There is a study made by Christophe Baufreton et al in U.S.A. It was a pilot study for carrying out to assess the feasibility and the clinical impact of a combined approach of cardiopulmonary bypass (CPB) with reduced anti-coagulation [1-5]. They used 45 consecutive patients dividing into two; undergoing coronary artery bypass using standard CPB with full anti-coagulation (activated clotting time, ACT>450 s) (group 1=23); or closed, heparin-coated CPB with low anti-coagulation (ACT>250 s) (group 2=22). The two groups compared with the measurements of the heparin, protamine, total postoperative blood loss, hemitropic decrease etc. They use the value of that measurement, similar to our study to compare two groups, but they used only one time values for the comparison which is not similar to us. But they did not use any regression model for this study. Data were gathered from the database used in their department and analyzed using the statistical software. SPSS (SPSS for Windows). They also got variable's mean ± standard deviation and they used univariate analysis, t-tests. Mann-Whitney test and Wilcoxon test for paired data. By using multivariate analyses, they found that group 2 is to be more protected than the group 1 against myocardial cellular injury.

There is another similar study in which multivariate statistical methods were used. The study made by B.O. Boehm et al. accepted 21 November 2001. The aim of the study is to compare the efficacy and safety of premixed insulin aspart (30% free and 70% protamine-bound. BIAsp 30) with human insulin premix (BHI 30) used in a twice-daily injection regimen in people with Type1 and Type 2 diabetes. The comparison of the primary endpoint, HbA_{1c} at 12 weeks, was based on a non-inferiority criterion in accordance with normal regulatory practice. For primary endpoint the main analysis of variance (ANOVA) model was used. Statistical programming was performed using SAS v6.11 (SAS Inst.) on a UNIX platform or S-plus v4.0 Release 3 for Windows.

In this study the blood compatibility of the PMEA-coated and uncoated hollow fibers were investigated using multivariate statistical methods. The patients were divided into two groups: PMEA-coated users and the uncoated users.

Then the two groups were compared by the values of some measurements in the base of blood cells and proteins (total bilirubin, direct bilirubin, erythrocyte, albumin, fibrinogen, leukocyte, total protein etc.). Our recent study CPB operations were achieved in five different stages named as T1-T5 [16-24]. Three treatment times were taken for comparison: baseline (T1), after protamine injection (T4) and intensive care (T5) for this statistically analysis. The measurements were additionally compared by one more step, age and the patient's were divided into four age groups. The significant correlation value was estimated and suggested as dependent variable. The other measurement which is in high correlation with dependent variable was depicted as independent variables in a regression model. All multivariate statistical analysis was made by SPSS for Windows v10.0. [6-10].

MATERIALS AND METHODS

Design of the study and analysis

First subjects of the analysis, deal with a set of issues that are resolved after data are collected but before the main data analysis is run. Careful consideration of these issues is time-consuming and sometimes it is common, for instance, to spend many days in careful examination of data prior to running the main analysis that, itself, takes about 5 minutes. But consideration and resolution of these issues before the main analysis are fundamental to a honest analysis of the data.

The first issues concern the accuracy with which data have been entered into the data file and consideration of factors that could produce distorted correlations. Next missing data, the bane of (almost) every researcher, are assessed and dealt with. Next many multivariate procedures are based on assumptions: the fit between our data set and the assumptions is assessed before the procedure is applied. Transformations of variables to bring them into compliance with requirements of analysis are considered. Outliers, cases that are extreme, create other headaches because solutions are unduly influenced and sometimes distorted by them. Finally, perfect or near-perfect correlations among variables can threaten a multivariate analysis.

Prior to analysis, number of patients, variables values and other information were examined through SPSS programs for accuracy of data entry, missing values and fit between their distributions and the assumptions of multivariate analysis. The variables are the values of total bilirubin, direct bilirubin, erythrocyte, albumin, fibrinogen, leukocyte, total protein, Aptt and pt taken in three different times (baseline, after protamine injection and intensive care).

There were 55 patients and related to these patients there were 27 variables. From these 27 variables with missing values on more than 5% of the cases were deleted. The number of variables was set to 21. Deleted variables are given below:

1. tbil values after protamine injection (16) .
2. dbil values after protamine injection (16).
3. Aptt values after protamine injection (17).
4. Pt values after protamine injection (17).
5. Aptt value in intensive care (4).
6. Pt value in intensive care (5).

For other variables missing values exchange with group mean and given in Table-1 in the result section. After that the values of skewness and kurtosis were examined and 8 variables out 27 variables were transformed with square root method. They were given below:

1. Aptt values of baseline.
2. Pt values of baseline.
3. Fibn. values of baseline.
4. Leuk. values after protamine injection
5. dbil. value in intensive care
6. tbil. value in intensive care
7. Alb. value in intensive care
8. tprot. value in intensive care

Two cases in the patients were univariate and multivariate outliers; so they were deleted and given below:

Univariate outliers and values;

1. Number 14 (dbil values of intensive care: 5.74).
2. Number 54 (fibn. values of baseline 4.302).
3. For the Mahaloanobis distance value of $p<0.001$ and df=20 (45.315) there were the multivariate outliers.

4. Number 14 (49.042).
5. Number 54 (49.874).

They all deleted and by this way the number of patients was set to 53.

After examining for accuracy of data entry, missing values and fit between their distributions and the assumptions of multivariate analysis; it was looked for the means of two groups through the variables. One-way ANOVA was used for this analysis and there are no significant difference between the two group (PMEA-coated and uncoated). Analysis of variance (ANOVA) is used to compare two or more means to see if there is any reliable difference s among them. Analysis of variance evaluates the differences among means relative to the dispersion in the sampling distributions. The null hypothesis is that $\mu_1 = \mu_2 = \ldots\ldots = \mu_k$ as estimated from $\hat{Y}_1 = \hat{Y}_2 = \ldots\ldots = \hat{Y}_k$ with k equal to the number of means being compared.

ANOVA (analysis of variance) is really a set of analytic procedures based on a comparison of two estimates of variance. One estimate comes from differences among scores within each group; this estimate is considered random or error variance. The second estimate comes from differences in group means and is considered a reflection of group differences or treatment effects plus error. If these two estimates of variance do not differ appreciably, one concludes that all of the group means come from the same sampling distribution of means differ more than expected, it is concluded that they were drawn from different sampling distribution of means, and the null hypothesis that the means are the same is rejected. Differences among variances are evaluated as ratios, where the variances associated with differences among sample mean is in the numerator and the variance associated with error is in the denominator. The ratio between these two variance forms an F distribution. F distributions change shape depending on degrees of freedom in both numerator and denominator of the F ratio. As can be seen from Table 2 no F value and p value are significant. Every p value is bigger than 005. Only for the value of intensive care fibrinogen value ($F(1.51)=8.661$. $p \leq 0.005$) and baseline Pt. value ($F(1.51)=9.315$. $p \leq 0.005$) the differences between coated and uncoated groups are significant. For this two value both the value of coated group's mean bigger than the uncoated mean.

Then the age variable recoded with 4 groups in order to examine the differences through the variables. The groups are given below:

age between 30-50 recoded as 1; age between 51-60 recoded as 2; age between 61-70 recoded as 3; age between 71-80 recoded as 4.

After examining for the means of two groups through the variables the differences through the age groups were analyzed. ONE_WAY ANOVA and Post Hoc tests were used for that analysis and no significant differences were found in the age (groups) as given by Table 3 in the result section.

Than the correlation for the variables were investigated. Correlation is the measure of the size and direction of the linear relationship between the two variables, and squared correlation is the measure of strength of association between them. Correlation is used to measure the association between variables, regression was also used. However, the equations for correlation and bivariate regression are very similar, as indicated in what follows. The correlation values bigger than 30 was taken.

Regression model

Whereas correlation is used to measure the size and direction of the linear relationship between two variables, regression is used to predict a score on one variable from a score on the other. In bivariate (two-variable) simple linear regression, a straight line between the two variables is found. The best-fitting straight line goes through the means of X and Y and minimizes the sum of the squared distances between the data points and the line. To find the best-fitting straight line for predicting Y from X, an equation is solved of the form.

$$\acute{Y} = A + BX$$

Where $\acute{Y}$ is the predict score. A is the value of Y when X is 0.00. B is the slope of the line (change in Y divided by change in X), and X is the value from which Y is to be predicted. The difference between the predicted and the observed values of Y at

each value of X represents errors of prediction or residuals. The best-fitting straight line is the line that minimizes the squared errors of prediction.

RESULTS AND DISSCUSION

TYPE VARIABLE

Taking type variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found as 0.249 (24.9%). The significant value for intensive care fibn. variable (sign. 0.005); for baseline pt variable (sign. 0.005). The interaction for both variable was found significant (sign. 0.001).

AGE VARIABLE

Taking the age variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found as 0.064 (6.4%). The value for baseline erit. variable was found significant (sign. 0.037).

Taking the variables which correlate with the chosen variable as independent and chosen one as dependent variable a regression model was made. The Blood cells results are given below.

BLOOD CELLS BEHAVIOUR

ERITHROCYTES VARIABLE

In baseline (T1): Taking the baseline alb. variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found 0.188 (18.8%). The significant value for baseline alb. variable (sign. 0.078); for age variable (sign. 0.036), for protamine injection erit. variable (sign. 0.012). The interaction for both variables was found significant (sign. 0.004).

After protamine injection (T4): Taking the protamine injection erythrocyte variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found 0.200 (20%).

Table 1. Result of other variables missing values exchange.

RESULT VARIABLES	Missing Values Replaced	Creating Functions
First Non-Miss (1). Last Non-Miss (1). Valid Cases (55) for all Creating Functions		
TYPEI_1	0	SMEAN(TYPEI)
PATIENT_1	0	SMEAN(PATIENT_NO)
AGE_1	9	SMEAN(AGE) BASETB_1
0_0	1	SMEAN(BASETBIL)
1. BASETBIL_1	0	SMEAN(BASETBIL)
2. BASEDBIL_1	0	SMEAN(BASEDBIL)
3. BASELEUK_1	0	SMEAN(BASELEUK)
4. BASEALB_1	0	SMEAN(BASEALB)
5. BASEERIT_1	0	SMEAN(BASEERIT)
6. BASETPROT_1	0	SMEAN(BASTPROT)
7. BASEAPTT_1	0	SMEAN(BASEAPTT)
8. BASEPT_2	0	SMEAN(BASEPT)
9. BASEFIBN_1	0	SMEAN(BASEFIBN)
10.PROT_LEUK_1	8	SMEAN(PRO_LEUK)
11.PRO_ALB_1	0	SMEAN(PRO_ALB)
12.PRO_ERIT_1	8	SMEAN(PRO_ERIT)
13.PRO_TPROT_1	0	SMEAN(PRO_TPROT)
14.PRO_FIBN_1	0	SMEAN(PRO_FIBN)
15.IC_TBIL_1	4	SMEAN(IC_TBIL)
16. IC_DBIL_1	4	SMEAN(IC_DBIL)
17. IC_LEUK_1	2	SMEAN(IC_LEUK)
18. IC_ALB_1	0	SMEAN(IC_ALB)
19. IC_ERIT_1	2	SMEAN(IC_ERIT)
20. IC_TPROT_1	0	SMEAN(IC_TPROT)
21. IC_FIBN_1	0	SMEAN(IC_FIBN)

The significant value for protamine injection tprot. variable (sign. 0.003) and for baseline erit. variable (sign. 0.016). The interaction for both variables was found significant (sign. 0.001).

In intensive care (T5): Taking the intensive care erit. variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables ; the adjusted R was found 0.210 (21.0%). The significant value for protamine injection erit. variable (sign. 0.001) and for intensive care tprot variable (sign. 0.285). The interaction for both variables was found significant (sign. 0.001).

LEUKOCYTES VARIABLE

In baseline (T1): Taking the baseline leuk. Variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found 0.234 (23.4%). The significant value for intensive care leuk. variable (sign. 0.722); for baseline leuk. variable (sign. 0.002) The interaction for both variable was found significant (sign. 0.001).

After protamine injection (T4): Taking the protamine injection leuk. variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found 0.427 (42.7%) The significant value for baseline leuk. variable (sign. 0.002) and for intensive care leuk. variable (0.001). The interaction for variables was found significant (sign. 0.001).

In intensive care (T5): Taking the intensive care leuk. variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found 0.310 (31.0%). The significant value for protamine injection leuk. variable (sign. 0.001) and for baseline leuk. variable (sign. 0.722). The interaction for both variables was found significant (sign. 0.001).

PT. VARIABLE

In baseline (T1): Taking the baseline pt. variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables ; the adjusted R was found 0.138 (13.8%). The interaction for type variable was found significant (sign. 0.004).

IN VITRO BLOOD CELLS BIOCOMPATIBILITY INVESTIGATIONS

At the first part of this study, erythrocytes and leukocytes were counted in blood samples taken from uncoated and PMEA-coated oxygenators at five different stage of CPB operation period. The average of these values was calculated and significant differences were observed between them. At the same time these differences also show agreement with our clinical observations such as less postoperative haemorrhage.

The average of counted platelet values was calculated. More platelet loss was observed when T1, T4 and T5 blood values were compared. It was also observed that loss of platelet was less when PMEA-coated circuits have been used. For oxygenator containing uncoated fibers, average loss of platelet was found. Clinical observations are suitable with each other. It was reported that less post-operative haemorrhage and decreasing in bleeding time can be explained by platelet agglutination. More thrombocyte adhesion was found on uncoated fiber surfaces compared with PMEA-coated fibers. At the same time, more platelet aggregation was observed in medium containing uncoated fibers. More and long-time post-operative haemorrhage was observed when uncoated circuits have been used. These bleeding values may also be explained with the same reason [11-13, 16-24].

BLOOD PROTEINS BEHAVIOUR

HUMAN SERUM ALBUMIN VARIABLE

In Baseline (T1): Taking the baseline alb. variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found 0.476 (47.6%). The significant value for baseline tprot. variable (sign. 0.001); for baseline fibn. variable (sign. 0.001). for baseline erit variable (sign. 0.236). for protamine injection fibn. variable (sign. 0.835). The interaction for both variable was found significant (sign. 0.001).

After Protamine Injection (T4): Taking the protamine injection alb. variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables;

Table 2. Results of ANOVA for between groups.

ANOVA		Sum of Squares		Mean Squares		F	Sig.
Creating Function	Between Groups	Within Groups	Total	Between Groups	Within Groups	-	-
df	1	51	52	-	-	-	-
SMEAN(BASETBIL)	3.5E-03	3.428	3.432	3.493E-03	6.722E-02	0.052	0.821
SMEAN(BASELEUK)	1.235	158.130	159.365	1.235	3.101	0.398	0.531
SMEAN(BASEALB)	3.1E-02	3.137	3.168	3.052E-02	6.151E-02	0.496	0.484
SMEAN(BAESERIT)	0.137	12.265	12.402	0.137	0.240	0.569	0.454
SMEAN(BASTPROT)	1.1E-04	11.002	11.002	1.097E-04	0.216	0.001	0.982
SMEAN(PRO_ALB)	0.221	6.451	6.671	0.221	0.126	1.744	0.192
SMEAN(PRO_ERIT)	4.0E-03	9.627	9.631	4.002E-03	0.189	0.021	0.885
SMEAN(PRO_TPROT)	6.7E-02	37.167	37.235	6.703E-02	0.729	0.092	0.763
SMEAN(PRO_FIBN)	9.7E-04	24.449	24.450	9.676E-04	0.479	0.002	0.964
SMEAN(ICLEUK)	3.907	424.588	428.494	3.907	8.325	0.469	0.496
SMEAN(ICERIT)	5.9E-03	12.306	12.312	5.949E-03	0.241	0.025	0.876
SMEAN(ICFINB)	2.659	15.656	18.315	2.659	0.307	8.661	0.005
NBASEAPTT	5.1E-02	4.303	4.355	5.147E-02	8.438E-02	0.610	0.438
NBASEPT	0.151	0.828	0.979	0.151	1.624E-02	9.315	0.004
NBASEFIBN	2.8E-03	1.069	1.072	2.831E-03	2.096E-02	0.135	0.715
NPROLEUK	0.100	10.556	10.656	0.100	0.207	0.483	0.490
NICTPROT	1.2E-02	0.810	0.822	1.211E-02	1.588E-02	0.763	0.387
NBASDBIL	2.8E-02	0.639	0.667	2.793E-02	1.253E-02	2.230	0.142
NICTBIL	2.5E-03	2.269	2.271	2.531E-03	4.449E-02	0.057	0.812
NICDBIL	6.2E-04	1.033	1.033	6.195E-04	2.025E-02	0.031	0.862
NICALB	1.9E-02	0.667	0.696	1.923E-02	1.328E-02	1.448	0.234

the adjusted R was found 0.091 (9.1%). The interaction for protamine injection tprot. variable was found significant (sign. 0.016).

In Intensive Care (T5): Taking the intensive care alb. variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found 0.280 (28%). The interaction for intensive care tprot. variable was found significant (sign. 0.001).

TOTAL PROTEIN VARIABLE

In Baseline (T1): Taking the baseline t.prot variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found 0.262 (26.2%). The interaction for baseline alb. variable variable was found significant (sign. 0.001).

After Protamine Injection (T4): Taking the protamine injection t. prot variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables ; the adjusted R was found 0.328 (32.8%) The significant value for protamine injection alb. variable (sign. 0.005) and for baseline fibr. variable (sign. 0.003); for protamine injection erit. variable (sign. 0.035). The interaction for both variable s was found significant (sign. 0.001).

In Intensive Care (T5): Taking the intensive care t.prot variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found 0.365 (36.5%) The significant value for protamine injection erit. variable (sign. 0.038) and for intensive care alb. variable (sign. 0.001); for intensive care erit. variable (sign. 0.735). The interaction for both variables was found significant (sign. 0.001).

FIBRINOGEN VARIABLE

In Baseline (T1): Taking the baseline fibr. variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found 0.553 (53.3%). The significant value for baseline alb. variable (sign. 0.046) and for intensive care fibr. variable (sign. 0.001); for protamine injection tprot. variable (sign. 0.001) and for protamine injection fibr. variable (sign. 0.009). The interaction for both variables was found significant 0.001.

After Protamine Injection (T4): Taking the protamine injection fibn. variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found 0.212 (21.2%). The significant value for baseline fibn. variable (sign. 0.005) and for baseline alb. variable (sign. 0.536). The interaction for both variables was found significant (sign. 0.003).

In Intensive Care (T5): Taking the intensive care fibn. variable as dependent variable and the variables which are correlate high with the dependent one; as independent variables; the adjusted R was found 0.397 (39.7%). The significant value for baseline fibn. variable (sign. 0.001) and for type variable (sign. 0.002).The interaction for both variable was found significant (sign. 0.001).

IN VITRO BLOOD PROTEINS BIOCOMPATIBILITY INVESTIGATIONS

At the second part of this study, blood proteins were also investigated on blood samples taken from patients at three different stages of CPB (T1, T4, T5) for uncoated and PMEA-coated oxygenators. For this purpose, albumin, fibrinogen and total protein results were investigated comparatively.

The average of total protein and albumin values were calculated. Similar trends can be observed for uncoated and for PMEA-coated oxygenators. Statistically significant change was observed for all samples taken from all patients at five different stages. Observed fluctuations of albumin between uncoated and PMEA-coated oxygenators are important for amount of total protein loss. These differences are in agreement with clinical observations. This result is also parallel with literature. Saito et al. have also reported that PMEA-coated circuits adsorb less amounts of proteins than uncoated circuits [14, 16-24].

Loss of fibrinogen was also calculated and fibrinogen loss was observed by comparing T1 and T5 blood values. It was also observed that amount of

fibrinogen lost was diminished when PMEA-coated circuits have been used. For oxygenetor containing uncoated fibers, average loss of fibrinogen was found. "Activated Partial Tromboplastine Time (APTT)" and "Protrombin Time (PT)" values shown similar trend with fibrinogen loss and these results is compatible with the literature [15]. As reported in literature, observed fibrinogen losses from patients operated by uncoated circuits cause long blood clothing time [11-13, 16-24].

CONCLUSION

All the data were entered into computer, with the SPSS for Windows, version 10.0. At the beginning of the analysis there were 55 patients and 27 variables (not including the age and type variables). The variables are the values of total bilirubin, direct bilirubin, erythrocyte, albumin, fibrinogen, leukocyte, total protein, Aptt and pt taken in three different times (baseline, after protamine injection and intensive care). All the cleaning acts and the gathered data became reliable for the analysis. It was looked for the missing data and also skewness, kurtosis values and after this we have 53 patients and 22 variables (type and age variables also including).

First of all. the difference value between the types (coated and uncoated) were examined. For the 22 variables there is only two. which shown difference between type of the operations. These variables are: Intensive care fibrinogen value and baseline Pt. value. Their F value and p value are significant. It was found that the intensive care fibrinogen value ($F(1.51)=8.661$. $p\leq0.005$) and baseline Pt. value ($F(1.51)=9.315$. $p\leq0.005$) the differences between coated and uncoated groups are significant. For this two value both the value of coated group's mean bigger than the uncoated mean. Non of the other values are significant for differences of the two type of the operations.

It was decided to look for differences based for the age. The patients had an age distribution of 30-77; so 4 age groups were made, 30-50; second 51-60; third 61-70 and last 70-80. The first group was 30-50. because there was only one patient between the ages of 30-40. The differences between the age groups. for the variables. Because there were only four sub-groups of age variable. post hoc tests were used for the analysis;

Table 3. One-way ANOVA test results for age groups.

ANOVA	Sum of Squares			Mean Squares		F	Sig.
Creating Function	Between Groups	Within Groups	Total	Between Groups	Within Groups	-	-
df	3	49	52	-	-	-	-
SMEAN(BASETBIL)	4.6E-02	3.385	3.432	1.548E-02	6.909E-02	0.224	0.879
SMEAN(BASELOC)	20.070	139.295	159.365	6.690	2.843	2.353	0.083
SMEAN(BASEALB)	0.222	2.946	3.168	7.384E-02	6.012E-02	1.228	0.310
SMEAN(BAESERT)	1.310	11.092	12.402	0.437	0.226	1.930	0.137
SMEAN(BASTPROT)	0.516	10.486	11.002	0.172	0.214	0.804	0.498
SMEAN(PRO_ALB)	0.373	6.298	6.671	0.124	0.129	0.968	0.415
SMEAN(PRO_ERIT)	0.262	9.369	9.631	8.718E-02	0.191	0.456	0.714
SMEAN(PRO_TPRO)	3.766	33.469	37.235	1.255	0.683	1.838	0.153
SMEAN(PRO_FIBR)	0.191	24.259	24.450	6.383E-02	0.495	0.129	0.942
SMEAN(ICLOC)	20.042	408.452	428.494	6.681	8.336	0.801	0.499
SMEAN(ICERIT)	0.482	11.830	12.312	0.161	0.241	0.666	0.577
SMEAN(ICFBIR)	0.351	17.964	18.315	0.117	0.367	0.319	0.812
NBASEAPTT	0.435	3.920	4.355	0.145	7.999E-02	1.813	0.157
NBASEPT	3.2E-02	0.447	0.979	1.070E-02	1.933E-02	0.553	0.648
NBASEFIB	4.9E-02	1.023	1.072	1.634E-02	2.087E-02	0.783	0.509
NPROLOC	1.180	9.477	10.656	0.393	0.193	2.033	0.121
NICTPR	5.9E-02	0.763	0.822	1.959E-02	1.557E-02	1.258	0.299
NBASDBIL	3.9E-02	0.628	0.667	1.285E-02	1.282E-02	1.002	0.400
NICTBIL	0.110	2.162	2.271	3.651E-02	4.412E-02	0.828	0.485
NICDBIL	3.6E-02	0.997	1.033	1.211E-02	2.035E-02	0.595	0.621
NICALB	1.5E-02	0.681	0.696	5.134E-03	1.390E-02	0.369	0.775

no differences were found between the age groups. There is neither significant F values nor the p values as seen in the in the tables of results.

Next correlations between the variables were analyzed; these correlations gave direction to the study with respect to the relationship between the variables. Only the significant correlations. according to both 0.01 and 0.05 reliability values and also larger than the strength 30. An SPSS computer program was used to find the correlations. There was a good screen of the relationship and correlations between the variables taken.

After examing the correlations. a regression model were made in order to see the pattern of correlated variables. In this regression model, a variable was taken as dependent variable and the other variables which correlated with the selected variable as dependent. All of the models established that the analysis were significant with both squared R. p and F values. In some regression models, only interaction effect was seen with the variables. These interactions were also given in the study. These results explain why the bleeding takes a long time, when uncoated oxygenators were used. Consequently, it can be said that PMEA-coating created a more hydrophilic surfaces for protein adsorption.

REFERENCES

1. B.G. Tabachick, L.S. Fidell, Using Multivariate Statistics, Fourth Edition, Northridge: Harper Collins, (2001).
2. A.L. Edwards (Translated by Selim Hovardaoğlu) (1995), Entering to Linear Regression and Correlation, Hatipoğlu press.
3. P. Newbold, (translated by Ümit Şenesen) (2001), Statistics for Economy and Science of Business, Second Eddition, Literature press.
4. Boehm et al., Premixed insulin aspart 30 vs. premixed human insulin 30/70 twice daily: a randomized trial in Type 1 and Type 2 diabetic patients, Diabetes UK, Diabetic Medicine, (2002) 393.
5. Christophe Baufreton et al., A combined approach for improving cardiopulmonary bypass in coronary artery surgery: a pilot study, Perfusion, (2002) 407.
6. B.G. Tabachick, L.S. Fidell, Using Multivariate Statistics, Fourth Edition, Northridge : Harper Collins, (2001).
7. A.L. Edwards (Translated by Selim Hovardaoğlu), Entering to Linear Regression and Corelation, Hatipoğlu press, (1995).
8. P. Newbold (translated by Ümit Şenesen), Statistics for Economy and Science of Business, Second Eddition, Literature press, (2001).
9. Boehm et al., Premixed insulin aspart 30 vs. premixed human insulin 30/70 twice daily: a randomized trial in Type 1 and Type 2 diabetic patients, Diabetes UK, Diabetic Medicine, (2002) 393.
10. Christophe Baufreton et al., A combined approach for improving cardiopulmonary bypass in coronary artery surgery: a pilot study, Perfusion, (2002) 407.
11. H.I. Lazar, X. Zhang, T. Hamasaki, C.A. Memmelo, P. Treanor, S. Rivers, G.S. Aldea, S.A. Bernard, R.J. Shemin, Annual Thorac Surgery, 63 (1997) 1701.
12. H. Guckenberger, B. Hacker, T. Hartman, T. Scheybani, Z. Wang, W. Wiegrabe, W. Baumeister, J. Vac. Sci. Technol., B 9 (1991) 1227.
13. R.J. Leatherbarrow, M. Stedman, T.N.C. Wells , J. Mol. Biol., 22 (1991) 361.
14. N. Saito, S. Motoyama, J. Sawamoto, International Society for Artificial Organs, 24 (2000) 547.
15. F. Ayhan, A. Yousefirad, H. Ayhan, Biocompatibility Investigation and Urea Removal from blood by Urease Immobilized HEMA Incorporated Poly(Ethylenglycoldi metacrylate) microbeads, App. Biomat. Sec. J. Biomed. Mat. Res., 64B (2003) 13.
16. M. Kocakulak, S. Günaydın, R. Saber, B. Farsak, N. Bingöl, C. Koçum, T. Sarı, Y. Zorlutuna, H. Ayhan, E. Pişkin, Investigation of Blood Proteins Adsorption on Coated And Uncoated Hollow Fiber Surfaces in Extracorporeal Circuits During Cardiopulmonary Bypass, The International Symposium of IEEE, 15-18 October 2001, İstanbul, Turkey.
17. M. Kocakulak, S. Günaydın, R. Saber, C. Koçum, N. Bingöl, T. Sarı, Y. Zorlutuna, H. Ayhan, Investigation of Blood compatibility of PMEA coated extracorporeal circuit. J. of Bioactive and Compatible Polymers, 17 (2002) 343.
18. U. Demirkılıç, E. Kuralay, H. Tatar, M. Kocakulak, C. Koçum, H. Ayhan, Clinical Evaluation and Blood Compatibility OF Coated and Flow Optimized Oxygenators, MEDICON July 2004 Conference, Italy.
19. M. Kocakulak, T. Özgürtaş, F. Ayhan, H. Ayhan, PMEA Coated Oxygenators Saves Red Blood Cells ın open heart surgery, The 11th International Biomedical Science and Technology Days, BIOMED2004, 6-10 September 2004, Ankara, Turkey.
20. M. Kocakulak, L. Durgun, F. Ayhan, H. Ayhan, Statistical Analysis of Blood Components Behavior on Various Extracorporeal Circuits, The 11th Internat. Biomed. Sci. &Tech. Days, BIOMED2004, 6-10 September 2004, Ankara, Turkey.

21. U. Demirkilic, M. Kocakulak, E. Kuralay, C. Koçum, H. Tatar, H. Ayhan, Coated and Flow Optimized Oxygenator: Clinical Evaluation and Blood Compatibility, J. Bioact.& Comp. Polym., 19 (2004) 395.
22. M. Kocakulak, C. Kocum, H. Ayhan, Scanning Tunnelling Microscopy of Immunologic Activation on Coated and Noncoated Oxygenator Fibers, The 27th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Shanghai, China, September 1-4, 2005.
23. M. Kocakulak, T. Özgürtaş, H. Ayhan, Effect of Hydrophilic Polymer Poly(2-Methoxyethyl acrylate) Coated Oxygenator on Haemolysis, J. Biomat. Sci., Polymer Edition, 17 (2006) 449.