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Standardization of single-handed jugular vein blood sampling technique for clinical pathology assessment in rats

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Abstract

The preferred site for blood collection for clinical pathology assessment depends on the volume and type of blood required, sampling intervals and state of consciousness of animals during sampling. Blood sampling from jugular vein from conscious rats has multiple advantages including simplicity, speed, the ability to incorporate repeated sampling (decreasing the need for satellite groups), collection in conscious animals and supports three Rs (Replacement, Reduction and Refinement). The objectives of this study were to standardise single handed jugular vein sampling in conscious male rats and compare the clinical pathology data generated, with samples obtained from fasted and anesthetized rats via retro-orbital bleeding, abdominal aorta and heart (ventricles). Forty-eight (48) rats were grouped into twelve rats each, per group per route. No biological or statistically significant differences were observed in any of clinical pathology parameters obtained by jugular vein blood collection in conscious rats compared to blood samples collected from other sampling routes under isoflurane anesthesia. It is concluded that single-handed blood sampling from jugular veins of conscious male Sprague-Dawley rats resulted in acceptable quality of samples for clinical pathology assessment. The single-handed jugular vein sampling technique can be considered as advantageous on welfare grounds as rats can be returned to the cages within minutes after sampling, consequently reducing the stress. Additionally, blood sampling from conscious rats enable the interpretation of the clinical pathology data without being influenced by anesthesia.

Key words: clinical pathology, blood sampling, conscious and anesthetized rats

Introduction

Rats are commonly used in non-clinical safety characterization of promising investigational medicines before first-in-man studies are conducted. Clinical pathology data can be useful in elucidating the mechanism of toxic responses observed in animals and extrapolation of adverse effects to humans. Blood samples used for measurement of clinical pathology endpoints can be collected from various routes namely, tail vein, retro-orbital venous plexus, sublingual vein, jugular vein, abdominal vena cava, abdominal aorta, and heart. Pre-analytical variables such as site of blood sampling, stress involved in handling of animals during sample collection and influence of anaesthetic agents employed may potentially influence the nature of the clinical pathology data and interpretation. The advantages and disadvantages of blood sampling from multiple routes under different conditions have been reported. Blood sampling from retro orbital plexus under anesthesia was reported to yield unpredictable and unacceptable variation/prolongation of PT and APTT clotting times for untreated male Wistar rats which was believed to be due to a local decrease of plasma coagulation factors, sample activation, coagulation factor consumption or release of local anticoagulant factors caused by traumatisation of the orbital sinus tissue during blood collection (Salemink, 1994). Blood collected from the retro orbital plexus and tail exhibited significant variations in white blood cell count, red blood cell count, hemoglobin, and hematocrit, and differences in leukocyte differential counts of lymphocytes and neutrophils when compared with other sites (Smith, 1968). Additionally, values of serum enzymes, cholesterol, calcium, phosphorus and creatinine were found to be higher in samples that were collected from retro orbital plexus and tail vein. Erythrocyte, haemoglobin and haematocrit were reduced in female rats exposed to 100% CO_2, methoxyflurane and isoflurane anesthesia. Sodium, inorganic phosphate, calcium and magnesium were reduced by methoxyflurane and isoflurane anesthesia, but increased by CO_2 concentrations (Deckardt, 2007).

Jugular vein sampling in conscious rats was recommended as the route of choice in rats as this method was reported to be simple, quick, and best of all, caused little to no stress to the rats (Zeleski *et al.,* 2011). This procedure was reported to allow blood collection by skilled phlebotomist at the continuous rate of 2 rats per minute, allows relatively larger volumes of collection, provide an option to collect blood samples of highest quality during interim time points, ability to collect the blood samples before the stress dependent alterations in the analytes because of the brevity of the restraint and more

importantly this method allows collection of specimens from an endothelial lined channel with minimal trauma which further makes them suitable for coagulation studies and similar analyses that require sample collection without tissue trauma (Meeks, 1989; Barry S Levine, 2014). It has been reported to be used as the route of blood collection for measurements of sensitive parameters such as cardiac troponins, a non-invasive marker of heart toxicity in rats (Reagan *et al.,* 2013). It was also reported that changes in the heart rate and blood pressure after jugular puncture was significantly lower when compared with tail vein within the first 2 h in conscious rats (Fitzner Toft *et al.,* 2006)

Considering the advantages of blood collection from jugular veins in conscious rats, the objectives of this study were to standardize the jugular vein blood collection in conscious male rats (Group 4), to determine the clinical pathology parameters and compare the data with other routes of blood collection viz. retro orbital venous plexus (Group 1), abdominal aorta (Group 2) and cardiac puncture (Group 3). To the author's knowledge, there were no published reports involving comparative clinical pathology data of blood samples collected from jugular veins of conscious rats with other conventional routes of blood sampling under anesthesia.

Materials and method

Animals

Animal activities were performed at Syngene Laboratory Animal Research, an AAALAC accredited facility, following Institutional Animal Ethical Committee approved animal test methods and standard operating procedures, and animal enrichment programs. Male Sprague-Dawley rats were obtained from Vivo Bio Tech Ltd. (Hyderabad, India). Rats were of 8–11 weeks of age, individually housed in stainless-steel cages, offered water *ad libitum*, and fed rodent diet (ALTROMIN 1314P) during the entire course of the experimental period. For the duration of the study, mean humidity (targeted mean range: 30 to 70%) and temperature (targeted range: 64 to 79° F) were maintained within acceptable ranges, and the room was on a 12-hour light/dark cycle. After a period of acclimation, rats were randomly assigned, using a computer-generated stratified randomization procedure based on current body weights such that all groups (12 rats per sampling group) had approximately equal mean body weights. Animals from which blood collected through retro orbital venous plexus were termed as group 1 animals; animals from which blood collected through abdominal aorta were termed as group 2 animals; animals from which blood collected through cardiac puncture were termed as group 3 animals; animals from which blood collected through jugular vein from conscious male rats were termed as group 4 animals. Following randomization and group assignment, all rats were given a unique permanent individual identification number. Rats were fasted for an approximate duration of 14 hours before blood sampling. Blood sampling from retro-orbital venous plexus, abdominal aorta and cardiac puncture were carried out under isoflurane anesthesia (5 %) using the vaporizer. The depth of the anesthesia was assessed by carrying out pedal reflex test. Blood sampling from jugular vein was carried out by manually restraining the rats. The jugular vein was located and the needle (23 gauge) was inserted into the region parallel to the vein. Once in the vein, a slight negative pressure was applied by pulling back the syringe plunger to allow the blood to be collected into the syringe.

Collection of blood samples

Approximately 300 to 400 µL of blood was collected from all animals from groups 1-4 into appropriately sized microtainer tubes (BD, containing K_2EDTA) and serum separator tubes for hematology and clinical chemistry, respectively. Hematology analysis was carried out using ADVIA 2120 hematology analyser (Siemens) using the in house standard operating procedures. Blood samples intended for clinical chemistry were centrifuged at 1200-1500 g for 10 - 15 minutes to harvest serum. Serum samples were visually inspected for hemolysis before analysis. Clinical chemistry analysis was carried out using Olympus AU400 automated analyser (Beckman Coulter). Group mean ratio of clinical pathology data was calculated by dividing the group mean of respective parameter from groups 1-3 by mean data from group 4. The data were subjected to statistical analysis (one-way ANOVA followed by Dunnett's Multiple Comparison test and test for equivalence of means of the clinical pathology parameters among routes of sampling) using GraphPad Prism 5 and statistically significant differences were reported. In addition to statistics, biological significance was also considered while interpreting the data. All the parameters investigated were expressed as arithmetic mean+SD and coefficient of variation. Additionally, absolute and percentage differences of means were calculated.

Results

Clinical observations and quality of samples

All the rats from all the groups were found to be healthy throughout the study period. No adverse clinical signs were observed in any of the groups during the course of the experiment. Blood samples collected from all the sampling sites revealed acceptable quality with respect to absence of clots and hemolysis.

Hematology

All the haematology values are presented in Table 1-3 and in Figure 1. Equivalence was established for the following parameters: HGB, MCHC, PLT, MONO and EOS. No equivalence was found for remaining hematology parameters tested. The absolute basophil value displayed the largest differences in haematology parameters in 2 or more sampling groups. The smallest differences occurred in the hemoglobin and MCHC parameters in 2 or more sampling groups. Coefficients of variation differences among the sampling sites greater than factor 1.5 were detected for HGB, HCT, MONO, BASO and RETIC.

A minimal decrease (0.7-0.8x) in WBC and absolute lymphocytes was observed in blood samples collected under isoflurane anesthesia in groups 1-3 when compared to samples collected from jugular vein (Group 4) in conscious rats. All other statistically significant alterations in group mean values observed were considered within normal biological variation and/or were not related to routes of sampling.

Clinical chemistry

All the clinical chemistry values are presented in Table 4-6 and in Figure 2. Equivalence was established only for serum albumin in any one of the sampling groups. No equivalence was found for remaining clinical chemistry parameters. The triglycerides and globulin values displayed the largest differences in clinical chemistry parameters in 2 or more sampling groups. The smallest differences occurred in the

sodium in 2 or more sampling groups. Coefficients of variation differences among the sampling sites greater than factor 1.5 were detected for AST and CREAT.

A minimal increase (1.2x) in serum total protein and serum globulin (1.2x) was observed in blood samples collected under isoflurane anesthesia in groups 1-3 when compared to samples collected from jugular vein (Group 4) in conscious rats. All other statistically significant alterations in group mean values observed were considered within normal biological variation and/or were not related to routes of sampling.

Discussion

The results from the present study confirmed the published literature data that blood sampling routes and experimental conditions of blood collection influence the clinical pathology data generated. Comparison of blood samples collected from retro orbital plexus, cardiac puncture and abdominal aorta with jugular vein sampling revealed equivalence for 6 out of 33 clinical pathology parameters. The concept of testing for equivalence was chosen in the present study in order to evaluate the comparability of different blood sampling techniques. Despite the lack of demonstration of equivalence for several clinical pathology parameters in this study, there were no biological significance of the values obtained among the different sampling routes. The extreme differences in the mean vales were observed for absolute basophils (retro orbital plexus and cardiac puncture) and triglycerides and globulin (abdominal aorta and cardiac puncture). Comparison of coefficients of variation of the clinical pathology parameters from different sampling routes indicated absence of any significant differences in general except for the parameters, HGB, HCT, MONO, BASO, RETIC, AST and creatinine where a factor of variation of $\geq$ 1.5 fold higher in samples collected under isoflurane anesthesia when compared to sampling through jugular veins in conscious rats.

A minimal decrease (0.7-0.8x) in WBC and absolute lymphocytes was observed in blood samples collected under isoflurane anesthesia when compared to samples collected from conscious rats. The potential influence of anesthesia on WBC parameters cannot be ruled out as WBC count in rats anesthetized with isoflurane anesthesia was reported to be lower than that of conscious animals (Nakatsu *et al.,* 2017). Potential influence of stress on circulating leucocytes is well documented (Dhabhar *et al*.,1995). There was a large decrease (45-50 %) in WBC number, accompanied by a decrease in lymphocyte number (50-60 %) and an increase in neutrophil number (10-30 %) in Sprague-Dawley rats as a consequence of animal restraint-related, epinephrine-mediated, early stress effects. Hence, minimal increase in the absolute WBC count, contributed by minimal increase in absolute lymphocytes observed in jugular vein sampling could not be attributed to stress.

Blood sampling from jugular vein in conscious rats offers multiple advantages over conventional sampling routes under anesthetized animals. Blood sampling from the jugular vein does not require the use of a warming device or the need to anesthetize animals. In addition, one other major benefit when sampling from the jugular vein using a manual restraining method is that the animal is removed from its cage, blood sampled and returned to its cage within minutes, consequently reducing the stress which could affect the physiological state of the animal and influence other variables attributed to the measured blood parameters which may be associated with the blood sampling methodology. This included effect associated with the use of anesthesia, warming cabinets or from eye damage caused by orbital sinus sampling. Unlike the blood sampling routes that demand sacrifice of animals to collect the blood, clinical pathology parameters of interest can be monitored in a given animal over a period of time using the jugular vein sampling. To sum up, comparison of clinical pathology parameters from jugular vein blood sampling in male Sprague–Dawley rats revealed similarity in the clinical pathology parameters when compared to blood sampling from conventional routes. It can be concluded that jugular vein blood sampling in conscious rats appears to be reliable, less painful and stressful (refinement), requires relatively lesser number of animals (reduction) and recommended to be used for clinical pathology data generation in male Sprague-Dawley rats.

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Table 1: Effect of sampling methods on hematology parameters (Jugular vein vs Retro orbital plexus)

Parameter	Unit	JV (n=12) Mean±SD	CV	ROP (n=12) Mean±SD	CV	Means ratio	Δ	Δ %	CV ratio	Equiv. limit	Equiv.
Red Blood Cell count (RBC)	Mcells/ul	7.96±0.316	4.0	8.82±0.358	4.1	1.11	0.86	10.80	1.0	±0.34	NO
Hemoglobin (HGB)	g/dl	15.4±0.37	2.4	16±0.29	1.8	1.04	0.6	3.90	0.8	±0.33	NO
Hematocrit (HCT)	%	48.6±1.35	2.8	51.6±1.22	2.4	1.06	3	6.17	0.9	±1.29	NO
Mean Cell Volume (MCV)	fL	61.1±2.52	4.1	58.5±1.75	3.0	0.96	-2.6	-4.26	0.7	±2.17	NO
Mean Cell HGB Concentration (MCH)	Pg	19.4±0.94	4.8	18.2±0.64	3.5	0.94	-1.2	-6.19	0.7	±0.80	NO
Mean Cell Hemoglobin Concentration (MCHC)	g/dl	31.7±0.64	2.0	31.1±0.59	1.9	0.98	-0.6	-1.89	0.9	±0.62	NO
Red Cell Distribution Width (RDW)	%	11.3±0.45	4.0	11.8±0.51	4.3	1.05	0.5	4.42	1.1	±0.48	NO
Absolute Reticulocytes (RETICS)	Mcells/ul	0.296±0.03	10.1	0.357±0.0458	12.8	1.2	0.061	20.61	1.3	±0.04	NO
Platelets (PLT)	Kcells/ul	995±173.9	17.5	921±195.3	21.2	0.93	-74	-7.44	1.2	±184.91	NO
White Blood Cells (WBC)	Kcells/ul	10.25±1.984	19.4	8.07±1.801	22.3	0.79	-2.18	-21.27	1.2	±1.89	NO
Neutrophils (NEU)	Kcells/ul	1.02±0.259	25.4	0.92±0.328	35.7	0.9	-0.1	-9.80	1.4	±0.30	NO
Lymphocytes (LYMP)	Kcells/ul	8.85±1.898	21.4	6.77±1.661	24.5	0.76	-2.08	-23.50	1.1	±1.78	NO
Monocytes (MONO)	Kcells/ul	0.16±0.038	23.8	0.15±0.059	39.3	0.95	-0.01	-6.25	1.7	±0.05	YES
Eosinophils (EOS)	Kcells/ul	0.08±0.035	43.8	0.12±0.026	21.7	1.51	0.04	50.00	0.5	±0.03	NO
Basophils (BASO)	Kcells/ul	0.03±0.009	30.0	0.02±0.011	55.0	0.63	-0.01	-33.33	1.8	±0.01	NO

SD = standard deviation; CV = coefficient of variation (%); Δ = absolute mean difference between the jugular vein (JV) and retro orbital plexus (ROP); Δ % = percentage mean value difference between JV and ROP

Table 2: Effect of sampling methods on hematology parameters (Jugular vein vs Abdominal aorta)

Parameter	Unit	JV (n=12) Mean±SD	CV	AA (n=12) Mean±SD	CV	Means ratio	Δ	Δ %	CV ratio	Equiv. limit	Equiv.
Red Blood Cell count (RBC)	Mcells/ul	7.96±0.316	4.0	8.4±0.267	3.2	1.05	0.44	-4.76	0.8	±0	NO
Hemoglobin (HGB)	g/dl	15.4±0.37	2.4	15.7±0.38	2.4	1.02	0.3	-1.88	1.0	±0.38	NO
Hematocrit (HCT)	%	48.6±1.35	2.8	49.2±1.28	2.6	1.01	0.6	-4.65	0.9	±1.32	NO
Mean Cell Volume (MCV)	fL	61.1±2.52	4.1	58.6±0.9	1.5	0.96	-2.5	0.17	0.4	±1.89	NO
Mean Cell HGB Concentration (MCH)	Pg	19.4±0.94	4.8	18.6±0.51	2.7	0.96	-0.8	2.20	0.6	±0.76	NO
Mean Cell Hemoglobin Concentration (MCHC)	g/dl	31.7±0.64	2.0	31.8±0.6	1.9	1	0.1	2.25	0.9	±0.62	YES
Red Cell Distribution Width (RDW)	%	11.3±0.45	4.0	11.7±0.55	4.7	1.04	0.4	-0.85	1.2	±0.50	NO
Absolute Reticulocytes (RETICS)	Mcells/ul	0.296±0.03	10.1	0.32±0.355	11.1	1.08	0.024	-10.36	1.1	±0.03	NO
Platelets (PLT)	Kcells/ul	995±173.9	17.5	979±109	11.1	0.98	-16	6.30	0.6	±145.12	YES
White Blood Cells (WBC)	Kcells/ul	10.25±1.984	19.4	7.46±1.049	14.1	0.73	-2.79	-7.56	0.7	±1.59	NO
Neutrophils (NEU)	Kcells/ul	1.02±0.259	25.4	0.78±0.242	31.0	0.76	-0.24	-15.22	1.2	±0.25	NO
Lymphocytes (LYMP)	Kcells/ul	8.85±1.898	21.4	6.3±0.791	12.6	0.71	-2.55	-6.94	0.6	±1.45	NO
Monocytes (MONO)	Kcells/ul	0.16±0.038	23.8	0.15±0.05	33.3	0.92	-0.01	0.00	1.4	±0.04	YES
Eosinophils (EOS)	Kcells/ul	0.08±0.035	43.8	0.11±0.039	35.5	1.35	0.03	-8.33	0.8	±0.04	NO
Basophils (BASO)	Kcells/ul	0.03±0.009	30.0	0.02±0.007	35.0	0.6	-0.01	0.00	1.2	±0.01	NO

SD = standard deviation; CV = coefficient of variation (%) ; Δ = absolute mean difference between the jugular vein (JV) and abdominal aorta (AA); Δ % = percentage mean value difference between JV and AA

Table 3: Effect of sampling methods on hematology parameters (Jugular vein vs Cardiac puncture)

Parameter	Unit	JV (n=12) Mean±SD	CV	CP (n=12) Mean±SD	CV	Means ratio	Δ	Δ %	CV ratio	Equiv. limit	Equiv.
Red Blood Cell count (RBC)	Mcells/ul	7.96±0.316	4.0	8.43±0.32	3.7	1.06	0.47	5.90	0.9	±0	NO
Hemoglobin (HGB)	g/dl	15.4±0.37	2.4	15.5±0.61	3.9	1.01	0.1	0.65	1.6	±0.50	YES
Hematocrit (HCT)	%	48.6±1.35	2.8	49.2±2.18	4.4	1.01	0.6	1.23	1.6	±1.81	NO
Mean Cell Volume (MCV)	fL	61.1±2.52	4.1	58.4±1.49	2.6	0.95	-2.7	-4.42	0.6	±2.07	NO
Mean Cell HGB Concentration (MCH)	Pg	19.4±0.94	4.8	18.4±0.32	1.7	0.95	-1	-5.15	0.4	±0.70	NO
Mean Cell Hemoglobin Concentration (MCHC)	g/dl	31.7±0.64	2.0	31.6±0.66	2.1	1	-0.1	-0.32	1.0	±0.65	YES
Red Cell Distribution Width (RDW)	%	11.3±0.45	4.0	11.8±0.34	2.9	1.05	0.5	4.42	0.7	±0.40	NO
Absolute Reticulocytes (RETICS)	Mcells/ul	0.296±0.03	10.1	0.359±0.07	19.4	1.21	0.063	21.28	1.9	±0.05	NO
Platelets (PLT)	Kcells/ul	995±173.9	17.5	881±159.90	18.1	0.89	-114	-11.46	1.0	±167.05	NO
White Blood Cells (WBC)	Kcells/ul	10.25±1.984	19.4	7.21±1.44	20.0	0.7	-3.04	-29.66	1.0	±1.73	NO
Neutrophils (NEU)	Kcells/ul	1.02±0.259	25.4	0.75±0.25	33.9	0.73	-0.27	-26.47	1.3	±0.26	NO
Lymphocytes (LYMP)	Kcells/ul	8.85±1.898	21.4	6.14±1.33	21.7	0.69	-2.71	-30.62	1.0	±1.64	NO
Monocytes (MONO)	Kcells/ul	0.16±0.038	23.8	0.11±0.05	43.6	0.69	-0.05	-31.25	1.8	±0.04	NO
Eosinophils (EOS)	Kcells/ul	0.08±0.035	43.8	0.09±0.02	23.3	1.08	0.01	12.50	0.5	±0.03	YES
Basophils (BASO)	Kcells/ul	0.03±0.009	30.0	0.02±0.01	45.0	0.58	-0.01	-33.33	1.5	±0.01	NO

SD = standard deviation; CV = coefficient of variation (%); Δ = absolute mean difference between the jugular vein (JV) and cardiac puncture (CP); Δ % = percentage mean value difference between JV and CP

Table 4: Effect of sampling methods on serum chemistry parameters (Jugular vein vs Retro orbital plexus)

Parameter	Unit	JV (n=12) Mean±SD	CV	ROP (n=12) Mean±SD	CV	Means ratio	Δ	Δ %	CV ratio	Equiv. limit	Equiv.
Aspartate Aminotransferase (AST)	U/l	162±13.80	8.52	148±23.4	15.81	0.9	-14	-8.64	1.9	NA	NA
Alanine Aminotransferase (ALT)	U/l	37±4.20	11.35	30±4.6	15.33	0.8	-7	-18.92	1.4	NA	NA
Alkaline Phosphatase (ALP)	U/l	276±72.50	26.27	214±36.2	16.92	0.8	-62	-22.46	0.6	±57.30	NO
Total Bilirubin (TBIL)	mg/dl	0.12±0.03	28.33	0.14±0.02	14.29	1.2	0.02	16.67	0.5	±0.03	NO
Direct Bilirubin (DBIL)	mg/dl	0.03±0.02	66.67	0.05±0	0.00	1.7	0.02	66.67	0.0	±0.01	NO
Blood urea nitrogen (BUN)	mg/dl	15±1.70	11.33	14±1	7.14	1	-1	-6.67	0.6	±1.39	NO
Creatinine (CREA)	mg/dl	0.33±0.02	5.15	0.38±0.026	6.84	1.1	0.05	15.15	1.3	±0.02	NO
Cholesterol (CHOL)	mg/dl	95±11.90	12.53	80±11.4	14.25	0.8	-15	-15.79	1.1	±11.65	NO
Triglycerides (TRIG)	mg/dl	56±17.70	31.61	43±12.5	29.07	0.8	-13	-23.21	0.9	±15.32	NO
Glucose (GLU)	mg/dl	107±14.40	13.46	93±10.8	11.61	0.9	-14	-13.08	0.9	±12.73	NO
Total Protein (TP)	g/dl	5.3±0.15	2.83	6.38±0.17	2.66	1.2	1.08	20.38	0.9	±0.16	NO
Albumin (ALB)	g/dl	3.3±0.12	3.64	3.4±0.09	2.65	1	0.1	3.03	0.7	±0.11	YES
Globulin (GLOB)	g/dl	1.9±0.08	4.21	2.9±0.11	3.79	1.5	1	52.63	0.9	±0.10	NO
Albumin/Globulin (A/G)	-	1.7±0.08	4.71	1.2±0.05	4.17	0.7	-0.5	-29.41	0.9	±0.07	NO
Calcium (CA)	mg/dl	10.4±0.21	2.02	10.3±0.16	1.55	1	-0.1	-0.96	0.8	±0.19	NO
Phosphorus (P)	mg/dl	9.7±0.55	5.67	8.6±0.32	3.72	0.9	-1.1	-11.34	0.7	±0.45	NO
Sodium (NA)	mmol/l	137±1.30	0.95	141±1.1	0.78	1	4	2.92	0.8	±1.20	NO
Potassium (K)	mmol/l	5.1±0.43	8.43	5.3±0.36	6.79	1	0.2	3.92	0.8	±0.40	NO
Chloride (CL)	mmol/l	97±1.50	1.55	103±1.7	1.65	1.1	6	6.19	1.1	±1.60	NO
Bicarbonate (HCO3)	mmol/l	22±1.70	7.73	20±1.8	9.00	0.9	-2	-9.09	1.2	±1.75	NO

SD = standard deviation; CV = coefficient of variation(%); Δ = absolute mean difference between the jugular vein (JV) and retro orbital plexus (ROP); Δ % = percentage mean value difference between JV and ROP

Table 5: Effect of sampling methods on serum chemistry parameters (Jugular vein vs Abdominal aorta)

Parameter	Unit	JV (n=12) Mean±SD	CV	AA (n=12) Mean±SD	CV	Means ratio	Δ	Δ %	CV ratio	Equiv. limit	Equiv.
Aspartate Aminotransferase (AST)	U/l	162±13.80	8.52	157±20.6	13.12	1	-5	-3.09	1.5	NA	NA
Alanine Aminotransferase (ALT)	U/l	37±4.20	11.35	28±3.3	11.79	0.8	-9	-24.32	1.0	NA	NA
Alkaline Phosphatase (ALP)	U/l	276±72.50	26.27	230±39.1	17.00	0.8	-46	-16.67	0.6	±58.25	NO
Total Bilirubin (TBIL)	mg/dl	0.12±0.03	28.33	0.15±0.02	13.33	1.3	0.03	25.00	0.5	±0.03	NO
Direct Bilirubin (DBIL)	mg/dl	0.03±0.02	66.67	0.04±0	0.00	1.4	0.01	33.33	0.0	±0.01	NO
Blood urea nitrogen (BUN)	mg/dl	15±1.70	11.33	14±1.7	12.14	1	-1	-6.67	1.1	±1.70	NO
Creatinine (CREA)	mg/dl	0.33±0.02	5.15	0.37±0.035	9.46	1.1	0.04	12.12	1.8	±0.03	NO
Cholesterol (CHOL)	mg/dl	95±11.90	12.53	80±12.5	15.63	0.8	-15	-15.79	1.2	±12.20	NO
Triglycerides (TRIG)	mg/dl	56±17.70	31.61	32±13.4	41.88	0.6	-24	-42.86	1.3	±15.70	NO
Glucose (GLU)	mg/dl	107±14.40	13.46	78±8.9	11.41	0.7	-29	-27.10	0.8	±11.97	NO
Total Protein (TP)	g/dl	5.3±0.15	2.83	6.2±0.19	3.06	1.2	0.9	16.98	1.1	±0.17	NO
Albumin (ALB)	g/dl	3.3±0.12	3.64	3.3±0.08	2.42	1	0	0.00	0.7	±0.10	YES
Globulin (GLOB)	g/dl	1.9±0.08	4.21	2.8±0.12	4.29	1.5	0.9	47.37	1.0	±0.10	NO
Albumin/Globulin (A/G)	-	1.7±0.08	4.71	1.2±0.04	3.33	0.7	-0.5	-29.41	0.7	±0.06	NO
Calcium (CA)	mg/dl	10.4±0.21	2.02	9.9±0.23	2.32	1	-0.5	-4.81	1.2	±0.22	NO
Phosphorus (P)	mg/dl	9.7±0.55	5.67	8.8±0.49	5.57	0.9	-0.9	-9.28	1.0	±0.52	NO
Sodium (NA)	mmol/l	137±1.30	0.95	146±0.7	0.48	1.1	9	6.57	0.5	±1.04	NO
Potassium (K)	mmol/l	5.1±0.43	8.43	5±0.21	4.20	1	-0.1	-1.96	0.5	±0.34	NO
Chloride (CL)	mmol/l	97±1.50	1.55	104±1.2	1.15	1.1	7	7.22	0.7	±1.36	NO
Bicarbonate (HCO3)	mmol/l	22±1.70	7.73	22±1.2	5.45	1	0	0.00	0.7	±1.47	NO

SD = standard deviation; CV = coefficient of variation (%) ; Δ = absolute mean difference between the jugular vein (JV) and abdominal aorta (AA); Δ % = percentage mean value difference between JV and AA

Table 6: Effect of sampling methods on serum chemistry parameters (Jugular vein vs Cardiac puncture)

Parameter	Unit	JV (n=12) Mean±SD	CV	CP (n=12) Mean±SD	CV	Means ratio	Δ	Δ %	CV ratio	Equiv. limit	Equiv.
Aspartate Aminotransferase (AST)	U/l	162±13.80	8.52	129±14.90	11.55	0.8	-33	-20.37	1.4	NA	NA
Alanine Aminotransferase (ALT)	U/l	37±4.20	11.35	30±3.80	12.67	0.8	-7	-18.92	1.1	NA	NA
Alkaline Phosphatase (ALP)	U/l	276±72.50	26.27	234±49.40	21.11	0.8	-42	-15.22	0.8	±62.03	NO
Total Bilirubin (TBIL)	mg/dl	0.12±0.03	28.33	0.15±0.02	10.67	1.3	0.03	25.00	0.4	±0.03	NO
Direct Bilirubin (DBIL)	mg/dl	0.03±0.02	66.67	0.03±0.00	0.00	1	0	0.00	0.0	±0.01	NO
Blood urea nitrogen (BUN)	mg/dl	15±1.70	11.33	14±1.00	7.14	0.9	-1	-6.67	0.6	±1.39	NO
Creatinine (CREA)	mg/dl	0.33±0.02	5.15	0.36±0.03	6.94	1.1	0.03	9.09	1.3	±0.02	NO
Cholesterol (CHOL)	mg/dl	95±11.90	12.53	74±8.30	11.22	0.8	-21	-22.11	0.9	±10.26	NO
Triglycerides (TRIG)	mg/dl	56±17.70	31.61	22±8.90	40.45	0.4	-34	-60.71	1.3	±14.01	NO
Glucose (GLU)	mg/dl	107±14.40	13.46	96±12.40	12.92	0.9	-11	-10.28	1.0	±13.44	NO
Total Protein (TP)	g/dl	5.3±0.15	2.83	6.2±0.18	2.90	1.2	0.9	16.98	1.0	±0.17	NO
Albumin (ALB)	g/dl	3.3±0.12	3.64	3.3±0.10	3.03	1	0	0.00	0.8	±0.11	NO
Globulin (GLOB)	g/dl	1.9±0.08	4.21	2.9±0.11	3.79	1.5	1	52.63	0.9	±0.10	NO
Albumin/Globulin (A/G)	-	1.7±0.08	4.71	1.2±0.07	5.83	0.7	-0.5	-29.41	1.2	±0.08	NO
Calcium (CA)	mg/dl	10.4±0.21	2.02	9.9±0.30	3.03	1	-0.5	-4.81	1.5	±0.26	NO
Phosphorus (P)	mg/dl	9.7±0.55	5.67	8.4±0.35	4.17	0.9	-1.3	-13.40	0.7	±0.46	NO
Sodium (NA)	mmol/l	137±1.30	0.95	146±1.00	0.68	1.1	9	6.57	0.7	±1.16	NO
Potassium (K)	mmol/l	5.1±0.43	8.43	4.9±0.24	4.90	1	-0.2	-3.92	0.6	±0.35	NO
Chloride (CL)	mmol/l	97±1.50	1.55	104±1.10	1.06	1.1	7	7.22	0.7	±1.32	NO
Bicarbonate (HCO3)	mmol/l	22±1.70	7.73	21±0.90	4.29	1	-1	-4.55	0.6	±1.36	NO

SD = standard deviation; CV = coefficient of variation (%); Δ = absolute mean difference between the jugular vein (JV) and cardiac puncture (CP); Δ % = percentage mean value difference between JV and CP

Figure 1: Group mean Summary of hematology parameters

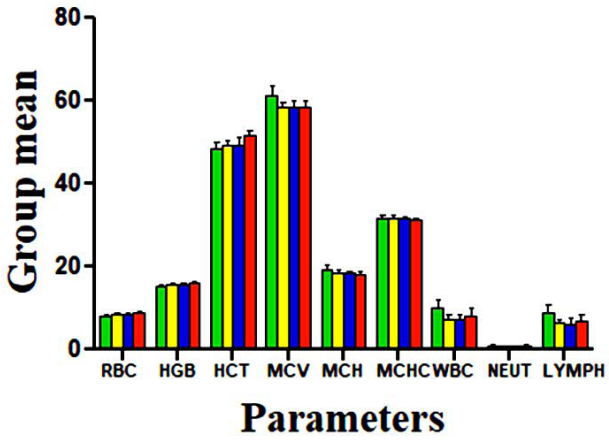

Figure 2: Group mean summary of clinical chemistry parameters