

Influence of tourniquet application on venous blood sampling for serum chemistry, hematological parameters, leukocyte activation and erythrocyte mechanical properties

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Abstract

Background: Venous blood sampling is usually performed using a tourniquet to help locate and define peripheral veins to achieve successful and safe venipuncture. Despite widespread usage of tourniquets for venipuncture by medical and laboratory staff, very few are aware of the effects of tourniquet application on laboratory parameters. In addition, definitive guidelines regarding when and how to use a tourniquet for blood sampling are lacking. The aim of the present study was to define the optimal sampling time after tourniquet removal to avoid adverse impact on laboratory analytes.

Methods: Blood oxygen and carbon dioxide partial pressure, pH, oxyhemoglobin saturation (satO₂), hematological parameters, serum electrolyte concentrations, erythrocyte deformability and aggregation, leukocyte activation and nitrite/nitrate concentrations obtained 180 s after tourniquet release were compared with baseline values for 10 healthy subjects.

Results: Blood gases, hematological parameters and serum electrolyte levels were not affected by the application and removal of a tourniquet. However, there were significant decreases in erythrocyte deformability at 90, 120, 180 s, and increases in erythrocyte aggregation at 5 and 30 s following removal of the tourniquet. A significant increase in granulocyte respiratory burst at 60 s was observed, confirming leukocyte activation due to application of the tourniquet. There were no significant alterations of blood nitrite/nitrate levels.

Conclusions: Our blood sampling technique which mimicked the application and release of a tourniquet indicated unaltered values for routine blood gases, hematological testing and serum electrolyte levels. Conversely, hemorheological measurements can be

affected. Therefore, it is strongly recommended that tourniquet application should be avoided during blood sampling or, if this is not possible, the procedure should be well standardized and details of the sampling method should be reported.

Clin Chem Lab Med 2009;47:769–76.

Keywords: blood sampling; hemorheology; leukocyte activation; tourniquet application.

Introduction

A tourniquet is widely used during blood sampling for facilitating visualization of the vein and to perform successful venipuncture. Most of the studies in this area have investigated changes in hematologic, biochemical, coagulation and rheologic properties of venous blood at different time points following tourniquet application, but these results cannot be easily adapted to a single standard procedure (1–4). Evidence from previous studies is that tourniquet application before blood sampling may affect some laboratory parameters (1, 4, 5). However, although the use of a tourniquet for blood sampling has been discussed in the literature (6–8), there still is lack of definitive information indicating the most appropriate timing for blood sampling when a tourniquet is used.

In routine practice, blood samples are usually obtained without loosening the tourniquet. Thus, blood withdrawal is very rapid and maintaining the needle inside the vessel lumen is made easier due to venous stasis. Alternatively, it has been recommended that for hemorheological studies, the tourniquet should be removed at least 5 s prior to the sampling of blood (9). However, a recent study indicated that significant alterations may be detected in red blood cell (RBC) deformability and aggregation 5–90 s after the removal of tourniquet (10). Surprisingly, RBC deformability was found to be increased (10), thus suggesting a role for nitric oxide (NO) in the alteration of RBC deformability (11).

The aim of the present study was to investigate the magnitude and duration of changes in blood gases, hematological parameters, serum electrolyte concentrations, hemorheological parameters, nitrite/nitrate content and granulocyte burst activity in blood samples obtained shortly after the restoration of venous flow following a brief occlusion model of tourniquet application. Blood sampling, including simulated tourniquet application, was performed with blood samples obtained consecutively for 3 min after restoration of venous blood flow. The primary goal was

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Received December 29, 2008; accepted March 24, 2009;
previously published online May 11, 2009

to define the optimal sampling time following tourniquet removal that did not affect some of the widely used clinical laboratory and hematological/hemorheological parameters.

Materials and methods

Study design

Healthy male subjects ($n=10$), aged between 25–30 years, were included in this study. Written informed consent was obtained and the study was approved by the local Ethics Committee. The venipuncture procedure for all subjects was performed by an anesthetist with the subject in a sitting position. A 20G catheter (BD Venflon™, Pro Safety, Becton Dickinson, Helsingborg, Sweden) was inserted into the right antecubital vein using a tourniquet. The tourniquet was removed following insertion of the catheter. After insertion of the catheter and removal of the tourniquet, a 30-min waiting period was observed to allow for stabilization. Following stabilization, blood pressure in the left arm was measured and diastolic pressure determined (Figure 1). Control blood samples were obtained from the catheter as described below (see Collection of blood samples). A pneumatic cuff was then applied to the right arm above the previously inserted catheter and venous stasis was imposed for 30 s by inflating the cuff at a pressure 20 mm Hg higher than diastolic blood pressure (DBP, see next paragraph). The cuff was then deflated to mimic removal of a tourniquet, and blood samples were obtained from the catheter at 5, 30, 60, 90, 120, 150, 180 s following deflation of the cuff. Blood oxygen and carbon dioxide partial pressures (pO_2 and pCO_2), pH, oxyhemoglobin saturation ($satO_2$) hematological parameters, serum electrolyte concentrations, RBC deformability and aggregation, and nitrite/nitrate concentrations were measured in all blood samples. However, granulocyte respiratory burst was determined only in the blood samples obtained at baseline (control) and at 60 and 180 s following cuff deflation.

The use of a cuff pressure 20 mm Hg above DBP was determined based upon a pilot study performed at the Akdeniz University Intensive Care Unit using a patient having a radial artery catheter inserted for blood pressure monitoring. Eight physicians or nurses, who were unaware of the study protocol, were asked to apply an elastic tourniquet on the patients arm with the arterial catheter. The arterial pressure wave shape observed on a monitor was deformed, but did not disappear after the tourniquet application. The same type of deformation was observed when a pneumatic cuff was applied and gradually inflated to the range of mean arte-

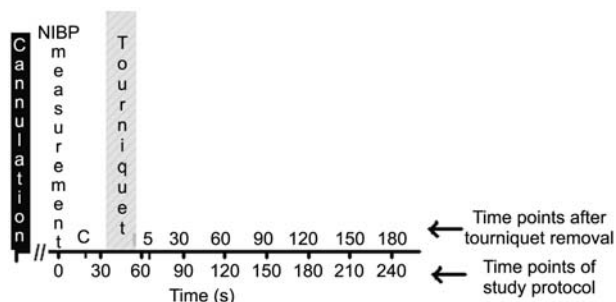


Figure 1 Experimental protocol and blood sampling times. The study was initiated with blood pressure measurement followed by blood sampling at defined times after tourniquet (i.e., pneumatic cuff) removal. NIBP, non-invasive blood pressure; C, control sample.

rial pressure for this patient (i.e., systolic blood pressure of 110–120 mm Hg, DBP of 55–60 mm Hg). Therefore, a cuff pressure ranging between systolic pressure and 20 mm Hg above DBP was selected for modelling application of the tourniquet. It should be mentioned that this standardized procedure resulted in a total occlusion of venous flow during the inflation of the cuff, but arterial flow was not abolished.

Collection of blood samples

Two separate blood samples were obtained at each time point:

1. Eight milliliter was collected at control sampling, and at 60 and 120 s following the deflation of the cuff. Four milliliter of blood was obtained at all other time points. Blood was collected into a syringe containing sodium heparin (15 IU/mL). These anticoagulated blood samples were used for analysis of blood pO_2 , pCO_2 , pH, $satO_2$, measurement of RBC deformability and aggregation, quantification of granulocyte respiratory burst and nitrite/nitrate concentration. Processing of the specimens was performed immediately after blood collection except for quantitation of nitrite/nitrate concentrations. Plasma was refrigerated ($-20^{\circ}C$) and used for quantitation of nitrite/nitrate following collection of specimens from all study subjects.
2. Four milliliter of blood was obtained using a syringe not containing anticoagulant, and immediately divided into two tubes: a) a K_2EDTA coated tube (BD Vacutainer Systems, Preanalytical Solutions, Bellerive Industrial Estate, Plymouth, UK) for analysis of hematological parameters. b) a serum separator tube containing gel to separate serum from RBC following centrifugation (Greiner Bio-One/VACUETTE®, Longwood, FL, USA). This was used for analysis of serum electrolytes. Samples were analysed within 1 h after collection.

Analysis of blood pO_2 , pCO_2 , pH, $satO_2$, hematological parameters and serum electrolyte concentrations

Analysis of blood pH, partial carbon-dioxide pressure (pCO_2), partial oxygen pressure (pO_2), oxygen saturation ($satO_2$), bicarbonate (HCO_3^-) content and base excess (BE) was performed by using a Stat Profile CCX blood gas analyzer (Nova Biomedical, Waltham, MA, USA).

RBC count, white blood cell (WBC) count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets, lymphocytes and neutrophils were assayed using a Beckman Coulter LH 780 (Beckman Coulter Inc, Fullerton, CA, USA). Serum sodium, potassium, chloride, calcium and magnesium were measured on a Roche/Hitachi Modular System P (Roche Diagnostics GmbH, Mannheim, Germany).

RBC deformability measurements

RBC deformability was measured as an elongation index (EI) at $37^{\circ}C$ for various fluid shear stresses by laser diffraction analysis using an ektacytometer (LORRCA, RR Mechatronics, Hoorn, The Netherlands). The principle of the system has been described elsewhere in detail (12). Briefly, a low hematocrit suspension of RBC in an isotonic viscous medium (4% polyvinylpyrrolidone 360 solution; MW 360 kDa) is sheared in a Couette system composed of a glass cup and a precisely fitting bob, with a gap of 0.3 mm between the cylinders. A laser beam is directed through the sample and the diffraction

pattern produced by the deformed cells is analyzed with a microcomputer. Based upon the geometry of the elliptical diffraction pattern, an EI is calculated as: $EI = (L - W) / (L + W)$, where L and W are the length and width of the diffraction pattern. At a constant shear stress, EI increases with RBC deformability.

EI values determined at the nine shear stress between 0.3–30 pascal (Pa) were used to calculate the shear stress required for half-maximal RBC deformation ($SS_{1/2}$) using Lineweaver-Burk analysis as described elsewhere (13). Briefly, shear stress – EI curves were linearized by plotting the reciprocal of EI as the function of the reciprocal of shear stress. The x-intercept of the line obtained by simple linear regression corresponds to the negative reciprocal value of shear stress causing $SS_{1/2}$. Thus, increased $SS_{1/2}$ values indicate impairment of RBC deformability.

RBC aggregation measurements

RBC aggregation was assessed using a custom-built photometric aggregometer, interfaced to a digital computer, that monitored light transmittance through the blood sample during the aggregation process (14). This device was used instead of the aggregation mode of the LORCA due to the smaller sample size (0.1 mL for aggregometer vs. 1 mL blood required for the LORCA). The shearing portion of the system consists of two parallel glass plates with a gap of 0.3 mm between them. A stepper motor, controlled by the computer, rotates one of these plates. The blood sample under investigation is placed between the glass plates, and is first sheared at 500 s^{-1} for 10 s to disperse pre-existing RBC aggregates. Following a sudden stop of the motor, the infrared light transmission through the blood sample is monitored for 10 s, integrated by the computer, and a dimensionless index calculated that increases with the extent of RBC aggregation. Measurements were performed in triplicate for each sample, and the mean of the three measurements used as the result. RBC aggregation was measured for cells in autologous plasma and for cells re-suspended in isotonic phosphate buffer saline (PBS) (290 mOsm/kg, pH=7.4) containing 0.5% dextran 500 (500 kDa, Sigma Chemical Co, St Louis, CA, USA) to determine the intrinsic tendency of RBCs to aggregate (15). Measurements were carried out at 37°C with the hematocrit of all samples adjusted to 0.4 L/L.

Quantification of granulocyte respiratory burst

The principle of the method has been described elsewhere (16). Briefly, granulocytes were isolated from whole blood using polysucrose density gradients (Histopaque 1119; Sigma Chemical Co.) as described previously (17). Isolated granulocytes were washed with and suspended in PBS at a cell count of $2.5 \times 10^6/\text{mL}$. The granulocyte suspension was then incubated in the dark at 37°C for 25 min in the presence of 10^{-6} mol/L 2', 7'-dichlorofluorescein diacetate (Catalogue No: D-6883; Sigma Chemical Co.). The fluorescence of dichlorofluorescein diacetate was determined in granulocyte suspensions ($10^6/\text{mL}$ PBS) using a Perkin Elmer LS-50 spectrofluorometer (Spectral Genomics, Boston, MA, USA) with 335 nm excitation and 655 nm emission wavelengths, the intensity of the fluorescence being directly related to the degree of activation of granulocytes, and therefore, granulocyte respiratory burst. Preparation of each specimen for quantitation of granulocyte respiratory burst took 1 h. Since every specimen was prepared in the same manner, the results were assumed to reflect the activation status of granulocytes at the time of specimen collection.

Quantification of nitrite/nitrate concentration

Nitrite/nitrate concentration in RBC suspensions was quantified using a colorimetric kit (No: 78001, nitrite/nitrate colorimetric assay kit; Cayman Chemical, Ann Arbor, MI, USA); the principle of the method has been previously described (18). Blood samples were centrifuged at 800 g for 6 min to isolate plasma. Proteins were removed from plasma samples by centrifugation at 100,000 g for 30 min at 4°C . Following centrifugation, the plasma was transferred to a 10-kDa cut-off filter (Millipore Corporation, Billerica, MA, USA) and re-centrifuged at 18,000 g for 120 min at 4°C . The resultant fluid was incubated with nitrate reductase and its cofactor at room temperature ($20\text{--}22^\circ\text{C}$) for 3 h. After a second incubation with Griess reactants (R_1 , R_2) for 10 min, absorbance was measured with a Thermo LabSystems Multiscan, spectrum-1500 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 540 nm. Nitrite/nitrate concentrations were calculated using a standard curve.

Statistical analysis

Values are presented as mean \pm SE of the mean. One-way analysis of variance (ANOVA) was used for repeated measures followed by Dunnetts Multiple Comparison post-hoc test. The level of statistical significance was set at $p < 0.05$. Analysis was performed using Graphpad Prism IV software (GraphPad Software Inc, La Jolla, CA, USA).

Results

Blood pO_2 , pCO_2 , pH, $satO_2$, hematological parameters and serum electrolyte concentrations

Blood pO_2 , pCO_2 , $satO_2$ and HCO_3^- values were not different in the samples obtained at 30 s intervals during the 3 min period following the restoration of venous flow. However, blood pH values were found to be decreased at 5 and 30 s following deflation of the pneumatic cuff. Values returned to baseline afterwards (Table 1).

Hematological parameters including RBC, WBC, platelet, lymphocyte and neutrophil counts, hemoglobin, hematocrit, MCV and MCHC were not significantly altered in any of the samples following cuff deflation (Table 2). Serum sodium, potassium, calcium and magnesium levels were also unaffected (Table 3).

RBC deformability

$SS_{1/2}$ values were found to be significantly increased at 90, 120 and 180 s after the pneumatic cuff was deflated but were not affected at earlier time points (Table 4). This indicated impaired RBC deformability.

RBC aggregation

RBC aggregation measured in autologous plasma was found to be increased significantly at 5 ($p < 0.05$) and 30 s ($p < 0.01$) after release of the pneumatic cuff. Values returned to baseline after 120 s (Table 4).

RBC aggregation measured in isotonic PBS containing 0.5% dextran 500 was not altered in any of the blood samples obtained after removal of the pneumatic cuff ($p > 0.05$) (Table 4).

Table 1 Blood gas analyses for samples obtained before the application of venous stasis and during the 180 s period following removal of the pneumatic cuff.

Parameter	Control	5 s	30 s	60 s	90 s	120 s	150 s	180 s
pH	7.389±0.005	7.370±0.005*	7.367±0.009*	7.377±0.005	7.378±0.005	7.380±0.008	7.378±0.007	7.382±0.006
pCO ₂ , mm Hg	43.65±2.06	45.97±1.33	45.16±1.48	44.83±1.42	44.08±1.45	44.14±2.08	44.88±1.88	43.52±1.36
pO ₂ , mm Hg	43.92±3.07	38.57±1.71	42.95±3.32	42.32±1.82	42.89±2.47	45.65±3.21	45.46±2.53	47.25±3.08
SatO ₂ , %	73.61±3.98	67.02±3.19	71.31±4.04	67.60±5.46	72.80±3.62	74.67±4.43	76.01±3.42	76.82±4.24
HCO ₃ ⁻ , mmol/L	26.76±1.05	27.07±0.70	26.93±0.68	26.84±0.65	26.48±0.63	26.40±0.80	26.84±0.77	26.25±0.60

Data are mean±SE, n = 10. pCO₂, carbon dioxide partial pressure; pO₂, oxygen partial pressure; satO₂, oxygen saturation; HCO₃⁻, bicarbonate. Difference from control: *p < 0.05.

Table 2 Hematology parameters for blood samples obtained before the application of venous stasis and during the 180 s period following removal of the pneumatic cuff.

Parameter	Control	5 s	30 s	60 s	90 s	120 s	150 s	180 s
Hematocrit, L/L	0.42±0.02	0.41±0.01	0.41±0.04	0.41±0.01	0.41±0.01	0.41±0.01	0.40±0.01	41.06±1.33
Hemoglobin, g/L	149.4±6.9	146.3±4.4	145.7±4.5	145.9±4.6	145.6±4.5	145.4±4.7	144.6±4.7	144.6±4.7
RBC count, × 10 ¹² /L	4.96±0.21	4.81±0.10	4.83±0.12	4.88±0.13	4.87±0.11	4.85±0.12	4.77±0.12	5.03±0.26
MCV, fL	85.16±1.45	85.35±1.38	85.45±1.42	85.40±1.47	85.52±1.45	85.61±1.42	85.46±1.42	85.30±1.39
MCHC, g/L	349.7±6.6	355.4±3.7	352.0±3.5	349.7±3.7	349.6±4.3	350.4±3.9	354.7±4.0	352.7±2.8
WBC count, × 10 ⁹ /L	6.92±0.39	6.94±0.35	6.99±0.34	6.95±0.38	6.99±0.39	6.84±0.33	6.64±0.28	6.70±0.33
Lymphocytes, × 10 ⁹ /L	2.34±0.18	2.41±0.19	0.38±0.16	2.38±0.19	2.38±0.22	2.39±0.20	2.28±0.18	2.32±0.18
Neutrophils, × 10 ⁹ /L	3.84±0.23	3.79±0.21	3.85±0.23	3.84±0.24	3.87±0.25	3.75±0.22	3.65±0.20	3.66±0.20
Platelet, × 10 ⁹ /L	249.8±19.2	264.2±20.5	261.3±21.6	261.2±20.0	261.4±19.5	253.9±19.4	263.2±20.7	255.8±18.0

Data are mean±SE, n = 10.

Table 3 Serum electrolytes and plasma nitrite/nitrate concentrations for blood samples obtained before the application of venous stasis and during the 180 s period following removal of the pneumatic cuff.

Parameter	Control	5 s	30 s	60 s	90 s	120 s	150 s	180 s
Sodium, mmol/L	141.0±0.63	141.7±0.63	141.6±0.84	140.7±0.36	142.2±0.68	141.5±0.77	140.8±0.66	141.0±0.71
Potassium, mmol/L	4.14±0.10	4.10±0.11	4.26±0.10	4.20±0.12	4.21±0.13	4.21±0.10	4.12±0.11	4.07±0.10
Chloride, mmol/L	106.1±0.93	106.2±0.78	105.6±1.09	105.6±1.04	106.4±0.97	105.5±0.63	105.8±1.24	106.1±0.80
Calcium, mmol/L	2.35±0.06	2.32±0.06	2.34±0.05	2.37±0.06	2.33±0.06	2.36±0.05	2.33±0.06	2.33±0.06
Magnesium, mmol/L	1.12±0.03	1.09±0.03	1.12±0.03	1.12±0.19	1.12±0.03	1.12±0.03	1.09±0.03	1.09±0.03
Nitrite/nitrate, μM	6.69±1.67	7.69±2.57	6.79±2.03	5.95±1.83	5.56±1.77	6.00±1.71	6.62±1.54	7.27±1.54

Data are presented as mean±SE, n = 10.

Table 4 Hemorheological parameters for RBC in autologous plasma or in an isotonic 0.5% dextran 500 kDa solution during the 180 s period following removal of the pneumatic cuff.

Parameter	Control	5 s	30 s	60 s	90 s	120 s	150 s	180 s
SS _{1/2} , Pa	3.43 ± 0.25	3.68 ± 0.26	3.58 ± 0.26	3.57 ± 0.26	3.82 ± 0.28 ^b	3.76 ± 0.24 ^a	3.69 ± 0.26	3.77 ± 0.32 ^a
RBC Aggregation index in autologous plasma	15.14 ± 0.87	18.30 ± 1.15 ^a	18.42 ± 1.03 ^a	16.39 ± 0.86	17.69 ± 0.68	14.65 ± 0.88	14.84 ± 0.95	15.03 ± 0.91
RBC aggregation index in 0.5% dextran 500	14.05 ± 1.43	15.43 ± 1.73	15.42 ± 1.51	15.43 ± 1.55	15.26 ± 1.60	14.70 ± 1.64	16.60 ± 2.22	13.76 ± 2.12

Data are mean ± SE, n = 10. Difference from control; ^ap < 0.05; ^bp < 0.01.

Granulocyte respiratory burst

Granulocyte respiratory burst increased at 60 s ($p < 0.01$) and returned to baseline values at 180 s ($p > 0.05$) following deflation of the cuff (Figure 2).

Nitrite/nitrate concentration

Plasma nitrite/nitrate concentrations did not change at any sampling time after deflation of the pneumatic cuff ($p > 0.05$) (Table 3).

Discussion

The results of the present study indicate that blood gases, hematological parameters and serum electrolyte concentrations were not significantly affected by temporary venous stasis when using a pneumatic cuff as a model for tourniquet use during blood sampling. These findings are in contrast with previous reports (1, 2). However, there were significant decreases in RBC deformability and increases in RBC aggregation following release of the pneumatic cuff. Granulocyte respiratory burst was significantly increased at 60 s after the restoration of venous flow, indicating activation following a short 30 s period of venous stasis. Finally, restoration of venous flow following 30 s of stasis did not cause significant alterations in blood nitrite/nitrate concentrations.

A decrease in pO_2 during tourniquet application that occludes venous return for 10 min has been previously reported (4). However, this 10 min duration is 20-fold longer than the 30 s used in this study. It should be noted that blood flow was restored before the start of sampling for both the current and previous study (4). The previous study reported a return of blood gas levels to baseline concentrations 60 s post-occlusion. This finding is consistent with our results at 60 s (Table 1). Blood pH was found to be slightly decreased at 5 and 30 s after cuff deflation (Table 1).

No significant changes in serum concentrations of sodium, potassium, chloride, calcium and magnesium were observed after release of the cuff. Renoe et

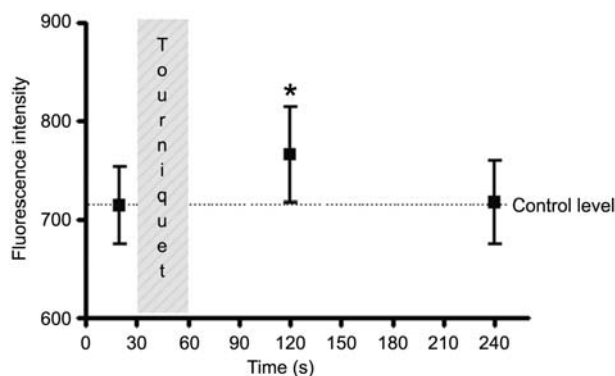


Figure 2 Granulocyte activation (i.e., respiratory burst) prior to and after release of pressure cuff. Fluorescence intensity was increased at 120 s and returned to control at 240 s. Data are mean ± SE, n = 10. Difference from control; *p < 0.05.

al. observed increased serum calcium concentrations at 1 min following the application of a pneumatic cuff (19). However, the duration of stasis was 3 min compared to 30 s used in this study. Furthermore, the effect of stasis was enhanced if the arm used for sampling was exercised. Continuous occlusion of venous flow following tourniquet use can alter electrolyte concentrations. Lippi et al. (2) report a significant decrease in potassium and a significant increase in calcium after 1 min of occlusion, a decrease of chloride at 3 min and unchanged sodium at both 1 and 3 min. However, Lippi et al. (2) obtained samples during venous stasis and not following restoration of blood flow.

Hematological parameters were not influenced by the 30 s period of venous stasis, regardless of the sampling following restoration of blood flow. The discrepancy between the results of the current study and the data from the previous studies exploring the effects of tourniquet application on clinical chemistry and hematological parameters may result from differences in the duration of venous stasis (tourniquet application) between studies (1, 2). Previous studies were performed with venous stasis following the application of a standardized external pressure of 60 mm Hg for 1 and 3 min. The external pressure was maintained until venipuncture and collection of blood were completed. However, in the current study, venous stasis was imposed for 30 s by inflating the cuff at a pressure 20 mm Hg higher than DBP, and blood samples were collected following deflation of the cuff.

Hemorheological changes after venous stasis, with or without arterial occlusion, have been reported previously (4, 5), with impairment of RBC deformability generally consistent with our findings. In the study by Forconi (4), whole blood filtration returned to baseline values in samples that were withdrawn 1 min after the release of cuff pressure. Subsequent measurements were not performed. However, in the current study, changes in RBC deformability started 90 s after restoration of venous flow, and did not return to baseline values at 3 min. In contrast, a recent study showed that RBC deformability was improved, rather than impaired, following removal of the tourniquet, with the alterations that were observed at 60 s no longer evident at 90 s (10). The discrepancy between the present study and the recent report (10) is most likely due to different approaches to stop blood flow. The pressure applied in the current study was well-defined (i.e., 20 mm Hg above diastolic), and thus was sufficient to occlude venous blood flow but not stop arterial inflow. Based upon the results of pilot studies to determine the appropriate pressure (see Study design above), we believe that our approach represents a more realistic model of tourniquet use during blood collection.

RBC aggregation in blood samples obtained at 5 and 30 s after restoration of venous flow was found to be increased in autologous plasma, but not in the standard 0.5% dextran 500 aggregating medium (Table 4). These findings indicate that cellular factors

contributing to RBC aggregation were not altered due to venous stasis. Rather, alterations in plasma composition (e.g., increased concentration of fibrinogen resulting from local fluid shifts) may be responsible for enhanced aggregation in plasma. Our findings of no changes between 60 and 180 s are in contrast to those of a previous report (10) which showed a decrease in RBC aggregation 90 s after the restoration of blood flow. As in previous studies different approaches used to stop blood flow may be responsible for these differences.

The mechanisms causing alterations in the parameters we studied following tourniquet application are not well known. Thus, we considered two factors that may be responsible for these changes.

(1) Neutrophil activation. Venous stasis may affect the number of marginated leukocytes and the number of blood cells adhering to venous endothelium. Leukocyte activation may increase with decreased venous blood flow velocity and shear rate (20). Also blood stasis and venous hypertension may lead to release of inflammatory mediators and growth factors that trigger the process of leukocyte recruitment and activation, migration, rolling and infiltration of the vessel wall (21). The results of the current study revealed an increase in granulocyte respiratory burst at 60 s which returned to baseline values at 180 s. It has been previously reported that activated leukocytes induce structural and functional alterations in neighboring RBC, including increased membrane lipid peroxidation and cell lysis, changes in RBC membrane skeletal proteins (e.g., cross-linking between spectrin and hemoglobin), and decreased RBC deformability (22–24). RBC aggregation has also been found to be enhanced by activated neutrophils, with the increase mediated by both proteolytic enzymes and oxygen free radicals (24). Thus the alterations of RBC deformability and aggregation in the current study may have resulted from leukocyte activation induced by venous stasis.

(2) Nitric oxide generation. NO is a well-known vasoactive mediator which regulates vascular tone, platelet aggregation, coagulation, fibrinolysis and leukocyte activation (25). Release of NO from vascular endothelium may be influenced by wall shear stress, intravascular stasis and ischemia-reperfusion injury (26, 27). In a previous study, plasma nitrate concentration was found to be significantly higher 5 min after reperfusion, compared to concentrations prior to ischemia (28). NO has also been shown to play a significant role in the regulation of RBC mechanical properties (11, 29), with a biphasic effect dependent on NO concentration (11). In addition to NO from external sources, NO generated within RBC has been suggested to influence deformability, based on observations related to significant effects of nitric oxide synthase (NOS) inhibitors (11). Recent studies have documented the activity of NOS in RBC. This NOS has properties similar to endothelial NOS (29, 30).

It has been suggested that NO may play a protective role in various patho-physiological processes characterized by impaired microcirculatory perfusion

(e.g., ischemia-reperfusion injury, sepsis, sickle-cell disease) (30). Therefore, it is tempting to suggest that alterations of NO generation may have played a role in hemorheological alterations following restoration of blood flow. However, in the current study, no significant change in the concentration of plasma nitrite/nitrate was observed, thereby excluding this possibility. Nevertheless, this finding should be interpreted cautiously as methods more sensitive than the Griess assay, which measures nitrite/nitrate concentration in the nM range, could indicate changes in NO activity (31).

In this study, blood samples were obtained from a catheter to collect serial blood samples and avoid multiple attempts at venipuncture after the release of the tourniquet. However, use of catheters to collect routine samples is not the normal procedure and this may influence several laboratory parameters. Extrapolating our findings to the design of routine blood collection procedures using needles should be one with caution. Another limitation of the current study was the lack of a parameter to evaluate platelet function. Platelet function might be changed after tourniquet release since shear stress is an important activator of platelets.

In conclusion, the results of this study indicate that blood sampling for the measurement of blood gases, hematological parameters or serum electrolyte concentrations can be performed with the application of a tourniquet for 30 s without any adverse effects on these measured parameters. However, hemorheological changes do occur following application of a tourniquet. Therefore, it is strongly recommended that tourniquet application should be avoided during blood collection for the investigation of hemorheological parameters. If this is not possible, the procedure should be standardized and details of the sampling method should be reported.

Acknowledgements

This study was supported by NIH Research Grants HL15722, HL 70595 and FIRCA IR03 TW01295, and by the Akdeniz University Research Projects Unit.

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