

Sampling time after tourniquet removal affects erythrocyte deformability and aggregation measurements

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Abstract. Venipuncture procedures are widely thought to influence biochemical, hematological or hemorheological measurements. In line with the preparation of the new Guidelines for the standardization of hemorheological measurement, we compared various blood rheological parameters (i.e., red blood cell deformability and aggregation indices) assessed in blood samples obtained after 5, 30, 60 and 90 s following the tourniquet removal and a blood sample obtained without applying a tourniquet (control sample). A slight but significant improvement in red blood cell (RBC) deformability after the removal of tourniquet compared to the control sample was observed. RBC deformability was maximal in the samples obtained 30 s after tourniquet removal and remained slightly higher than the control in the following samples (at 60 and 90 s after tourniquet removal). The aggregation index (AI) decreased with time after tourniquet removal reaching significantly lower values than the control at 90 s after tourniquet removal. This finding was supported by a greater half time for RBC aggregation in the samples obtained 60 and 90 s after tourniquet removal. In conclusion, this study revealed that RBC deformability and aggregation might be significantly altered in the samples obtained after the application and removal of a tourniquet, as a part of the blood sampling procedure. Recommendation “remove the tourniquet at least 5 s prior to the start of blood sampling” may need to be revised.

Keywords: Red blood cell deformability, red blood cell aggregation, methodology, venipuncture procedures, blood sampling

1. Introduction

Several methodological studies have investigated the influence of pre-analytical factors on various biochemical and hematological parameters, because it may produce spurious and clinically misleading biases in the measurements. One important methodological key point seems to be related to the venipuncture procedures. Usually, a tourniquet is applied during venipuncture to locate the vein to be sampled. Lippi et al. [9,10] demonstrated that tourniquet time may strongly affect the values of routine hematological and coagulation parameters. Leppanen et al. [8] reported higher serum potassium in the

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first filled tube after phlebotomy than in the following one. Rosenson and Tangney reported significant increment in plasma viscosity in the samples obtained 4–5 min after the application of a tourniquet [11]. Furthermore, the studies reported by Forconi showed that whole blood viscosity and whole blood filterability were altered within 3 min of application of a tourniquet [5]. The guidelines on blood rheology set by the expert panel of International Committee for Standardization in Haematology stated that:

a tourniquet should be used only to allow localization of the vein and insertion of the needle; the tourniquet must be removed for at least five seconds prior to actual blood withdrawal [3].

Therefore, in practice, usually the samples are obtained shortly after the removal of the tourniquet. In a preliminary study on healthy human male subjects, we investigated RBC aggregation and deformability in two separate samples obtained one after the other following the removal of a tourniquet and observed that both aggregation and deformability parameters were significantly different in the two samples (unpublished observations). No significant alterations in routine hematology parameters accompanied these hemorheological changes. Based on these preliminary observations and as a part of the preparation of the new Guidelines for the standardization of hemorheological measurement [2], we further assessed blood rheological parameters with serial samplings following the removal of tourniquet. The aim of these experiments was to find the optimal sampling time after tourniquet removal avoiding interference and clinical biases on blood rheological parameters.

2. Materials and methods

2.1. Study design

The study was conducted on healthy, male volunteers, after giving their informed written consent. The experiment included 17 subjects and was conducted on two separate laboratories involving 10 subjects in the Department of Physiology of Akdeniz University Faculty of Medicine (Antalya, Turkey) and 7 subjects in the Department of Physiology of the University of the French West Indies (Pointe-à-Pitre, Guadeloupe). Blood samples were obtained into four 10 ml vacutainer containing ethylenediaminetetraacetic acid (EDTA; 1.5 mg/ml) after 5, 30, 60 and 90 s following the tourniquet removal on one arm. The application time of the tourniquet was below 1 min for all samplings. Another sample was obtained from another antecubital vein on the other arm without applying a tourniquet, to serve as the control. All subjects were sampled in sitting position by two experienced technicians.

2.2. Hemorheological measurements

All the measurements were performed by handling the tubes in random order. RBC deformability (elongation index, EI) was measured at various fluid shear stresses by laser diffraction analysis using an ektacytometer at 37°C (LORRCA, RR Mechatronics, Hoorn, The Netherlands). The principle of the system was described elsewhere in detail [7]. EI values were determined at nine shear stresses ranging from 0.30 to 30 Pa. The EI values were presented as a function of shear stress and Lineweaver–Burk plots were obtained using GraphPad Prism 5 software for determination of the shear stress required for half-maximal deformation ($SS_{1/2}$) [1]. Increased $SS_{1/2}$ values represent a decreased RBC deformability.

The LORRCA was also used to determine various indices of both static and kinetic parameters of the RBC aggregation such as: RBC aggregation index (AI), aggregation half time ($t_{1/2}$) and the minimal shear rate to prevent aggregation ($\gamma_{T_{min}}$) [4,6].

2.3. Statistical analyses

Values are presented as means \pm standard error of the mean. A one-way analysis of variance (ANOVA) for repeated measures was used. Tukey post-hoc test was used when necessary to localize the difference. Statistical significance was established at $\alpha = 0.05$. Analyses were conducted using Statistica (v. 5.5, Statsoft, USA).

3. Results

3.1. RBC deformability

RBC EI measured in the samples following the tourniquet application and removal were found to be higher than the control values measured in the samples obtained without applying a tourniquet (Table 1). EI reached a maximum value in the samples obtained 30 s after the removal of the tourniquet and slightly decreased from that point in the following samples (at 60 and 90 s after the tourniquet removal). However, EI measured at shear stresses above 3 Pa were still significantly higher than control after 90 s. Despite these significant alterations in EI, $SS_{1/2}$ values were not significantly altered, although a slight decrement was observed starting with the first sample obtained 5 s after the tourniquet removal (Fig. 1).

3.2. RBC aggregation

Figure 2 shows that AI decreased with time after the tourniquet removal, the index measured at 90 s being significantly lower than control value. This finding was supported by $t_{1/2}$ values, indicating that RBC aggregation was significantly slower in the samples obtained 60 and 90 s after the removal of tourniquet (Fig. 3). Finally, disaggregation shear rate ($\gamma_{T_{min}}$) exhibited a minimum at 30 s and then gradually increased towards the control value in the following samples (Fig. 4).

Table 1

Comparisons of elongation index (EI) measured in the blood samples obtained at 5–90 s after the removal of a tourniquet and the control sample obtained without applying a tourniquet

| SS (Pa) | Control | 5 s | 30 s | 60 s | 90 s |
|---------|-------------------|--------------------|---------------------|--------------------|---------------------|
| 0.30 | 0.031 \pm 0.003 | 0.031 \pm 0.002 | 0.033 \pm 0.003 | 0.032 \pm 0.003 | 0.032 \pm 0.003 |
| 0.87 | 0.065 \pm 0.004 | 0.068 \pm 0.003 | 0.070 \pm 0.004* | 0.069 \pm 0.004 | 0.069 \pm 0.004 |
| 0.95 | 0.141 \pm 0.004 | 0.146 \pm 0.003 | 0.147 \pm 0.005* | 0.146 \pm 0.005 | 0.146 \pm 0.005 |
| 1.69 | 0.239 \pm 0.005 | 0.246 \pm 0.004 | 0.246 \pm 0.005* | 0.244 \pm 0.005 | 0.244 \pm 0.005 |
| 3.00 | 0.339 \pm 0.004 | 0.345 \pm 0.004 | 0.347 \pm 0.004* | 0.344 \pm 0.005 | 0.347 \pm 0.004* |
| 5.33 | 0.429 \pm 0.003 | 0.434 \pm 0.002 | 0.435 \pm 0.003* | 0.433 \pm 0.004 | 0.435 \pm 0.003* |
| 9.48 | 0.494 \pm 0.002 | 0.499 \pm 0.002* | 0.499 \pm 0.002* | 0.498 \pm 0.003 | 0.498 \pm 0.002* |
| 16.91 | 0.543 \pm 0.002 | 0.547 \pm 0.002* | 0.548 \pm 0.002** | 0.546 \pm 0.003 | 0.547 \pm 0.002* |
| 30.00 | 0.578 \pm 0.002 | 0.581 \pm 0.002* | 0.583 \pm 0.002** | 0.581 \pm 0.002* | 0.582 \pm 0.002** |

Values are means \pm SEM.

SS – shear stress.

Difference from control: * $p < 0.05$, ** $p < 0.01$.

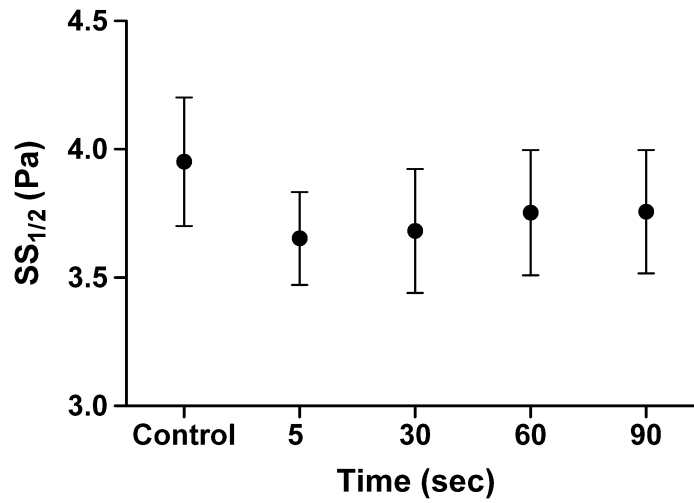


Fig. 1. Shear stress required for half-maximal deformation ($SS_{1/2}$) of red blood cell in the control sample and the samples obtained at various sampling times after the removal of tourniquet (5, 30, 60 and 90 s).

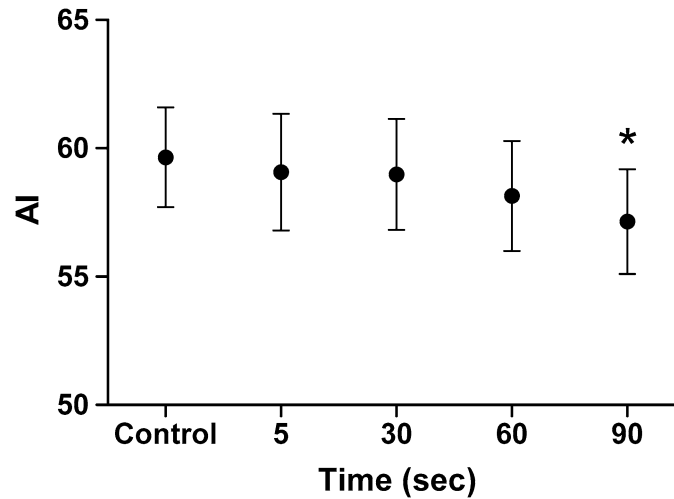


Fig. 2. Aggregation index (AI) in the control sample and the samples obtained at various sampling times after the removal of tourniquet (5, 30, 60 and 90 s), “*” significant difference from the control tube; $p < 0.05$.

4. Discussion

This study revealed that RBC deformability and aggregation might be significantly altered in the samples obtained after the application and removal of a tourniquet, as a part of the blood sampling procedure.

It has been previously discussed that venous stasis alone or together with arterial occlusion may induce alterations in blood rheology parameters, including plasma and whole blood viscosities and whole blood filterability [5,11]. Forconi reported a return towards the control value 1 min after the removal of tourniquet following a 10 min occlusion of venous circulation [5].

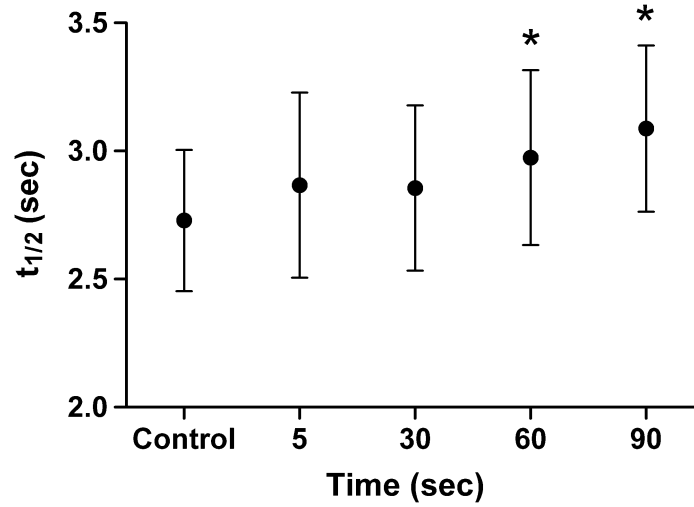


Fig. 3. Aggregation half time ($t_{1/2}$) in the control sample and the samples obtained at various sampling times after the removal of tourniquet (5, 30, 60 and 90 s), “*” significant difference from the control tube; $p < 0.05$.

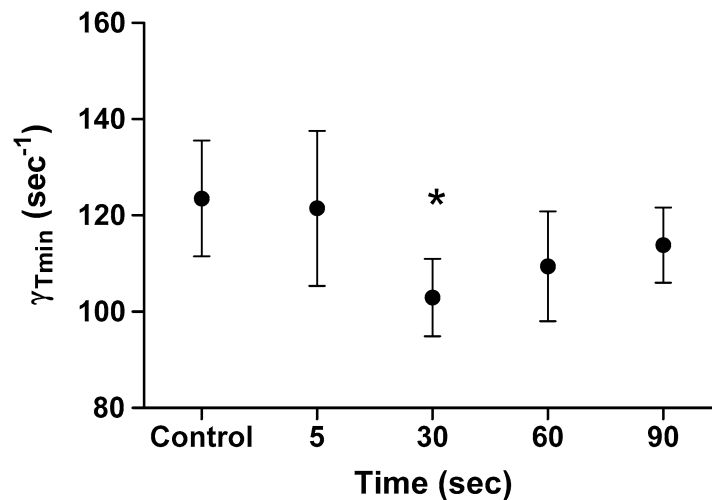


Fig. 4. Minimal shear rate to prevent aggregation (γ_{Tmin}) in the control sample and the samples obtained at various sampling times after the removal of tourniquet (5, 30, 60 and 90 s), “*” significant difference from the control tube; $p < 0.05$.

The usual sampling procedure for measuring hemorheological parameters include the removal of the tourniquet, as recommended by the “Guidelines for measurement of blood viscosity and erythrocyte deformability” set by the International Committee for Standardization in Haematology [3]. However, the results of this study suggest that there might be significant alterations that may continue to progress, even after 90 s following the removal of tourniquet. The design of this study did not include the measurements during the application of the tourniquet; therefore, the results are not directly comparable to the studies mentioned above. But, the current results seem to reflect the practice more closely, in compliance with the recommendations in the Guidelines mentioned above [3].

Rosenson and Tangney investigated the effect of the pressure applied during the tourniquet time and concluded that the tourniquet pressure is not an important factor for the samples obtained during its

application [11]. However, it may affect the alterations of blood rheology observed after the removal of the tourniquet, determining the mechanisms of alterations in blood rheology. It has been proposed that increased plasma viscosity during the application of tourniquet might have resulted from the transudation of plasma water due to venous stasis [11]. This may also lead to increased hematocrit levels but Forconi reported no significant alterations in hematocrit levels in the samples obtained after a venous stasis [5]. Our preliminary observations on the sequential samples obtained after the brief application and removal of a tourniquet did not indicate an alteration in hematocrit levels (unpublished observations).

The application of tourniquet might also be accepted as a brief ischemia and reperfusion afterward. Forconi reported decreased pO_2 values during tourniquet application, even at pressures sufficient only to occlude venous return [5]. This brief hypoxia may trigger mechanisms that may affect blood perfusing the microcirculation in the affected limb. This effect might be manifest in the samples obtained after restoring the venous return, i.e., after the removal of tourniquet. The mechanisms of this effect await for further investigations.

It is important to note that alteration in RBC aggregation continues to be more effective 90 s after the removal of the tourniquet. Therefore, it seems to be necessary to re-consider the recommendations on the “removal of the tourniquet at least 5 s prior to the sampling”. That might be a better idea to do the sampling immediately after the insertion of the needle, as soon as possible after the application of the tourniquet, in order to avoid the changes that may occur after the removal.

The effects of pre-analytical factors, including the blood sampling procedures, on measured biological parameters are of primary importance, because they may produce clinical biases in the measurements leading to misinterpretation. Development of hemorheological methods, enabling more accurate measurements increased the importance of the attention to sampling procedures, as slight alterations might become manifest using these methods.

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