Scintigraphic method for evaluating reductions in local blood volumes in human extremities

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We introduce a new method for evaluating reductions in local blood volumes in extremities, based on the combined use of autologue injection of 99mTc-radiolabelled erythrocytes and clamping of the limb blood flow by the use of a tourniquet. Twenty-two healthy male volunteers participated in the experiment. Evaluation of one versus two scintigraphic projections, trials for assessment of the reproducibility, a comparison of the scintigraphic method with a water-plethysmographic method and registration of the fractional reduction in blood volume caused by exsanguination as a result of simple elevation were carried out. No significant differences between results obtained by the use of one or two scintigraphic projections were found. The between-subject coefficient of variation was 14% in the lower limb experiment and 11% in the upper limb experiment. The within-subject coefficient of variation was 6% in the lower limb experiment and 6% in the upper limb experiment. We found a significant relation ($r = 0.42$, $p = 0.018$) between the results obtained by the scintigraphic method and the plethysmographic method. In fractions, a mean reduction in blood volume of $0.49 \pm 0.14$ (2 SD) was found after 1 min of elevation of the lower limb and a mean reduction of $0.45 \pm 0.10$ (2 SD) after half a minute of elevation of the upper limb. We conclude that the method is precise and can be used in investigating physiologic and pathophysiologic mechanisms in relation to blood volumes of limbs not subject to research previously.

Key words: Exsanguination; gravitation; haemodynamics; surgery; tourniquets

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The venous system in the extremities has an important role in the preload of the heart and it has a function as a blood reservoir [1]. The system is regulated by the sympathetic nerves and is influenced by gravity, arteriovenous resistance, hormones, the muscle pump, the venous valves, the respiratory pump, the abdominal pressure, and the suction effect of the heart [2, 3]. A wide spectrum of clinical routines is based on these mechanisms. For example, compression bandages are used in the prevention of deep venous thrombosis and for the treatment of deep venous insufficiency [4]. Trendelenburg’s manoeuvre is used to exsanguinate limbs in different types of shocks and before the application of a tourniquet in limb
surgery [5, 6]. Some of these procedures, however, have never been evaluated. So far, evaluation of changes in blood volumes and flow in the extremities has mainly been based on strain gauge, water and impedance plethysmographic methods [2, 7–16]. However, scintigraphic methods using 99mTc-labelled erythrocytes in combination with venous occlusion have also been introduced [17–20]. All of these methods have been based on venous occlusion, with the drawback of not being able to evaluate decreasing blood volumes. The purpose of our study was to establish a method for measuring limb blood volume changes as a result of physiological exsanguination. The new method is based on the combined use of 99mTc-labelled erythrocytes [21] and a tourniquet for clamping the residual blood volume.

MATERIALS AND METHODS

Lower limbs – procedure

Twelve healthy male volunteers with a mean age of 29 years (range 24–39) had autologue injection of 99mTc-radiolabelled erythrocytes with a mean radioactivity of 735 MBq (range 525–880). Radiolabelling of erythrocytes was done in accordance with the procedure presented by Pavel et al. [22]. Each subject was placed supine with the crura aligned in a frame in order to minimize variation of positioning (Fig. 1). The leg was then aligned with the foot and crus within the field-of-view of a double-headed gamma camera equipped with low-energy general-purpose collimators and interfaced with a dedicated computer (Maxxus, Star 4000i, GE Medical Systems, Wisconsin, Ill, USA). To ensure reproducible positioning of a region of interest (ROI) for subsequent integration of radioactivity, a 57Co source was placed 5 cm distal to the proximal demarcation of the right tibia. As ROI, we took the smallest rectangle that included the right foot and part of the right crus distal to the marking. The same ROI was used for all measurements in each subject.

Lower limbs – scintigraphic projection

Counts were obtained from both heads of the gamma camera, i.e., from both the anterior and posterior projections of the right lower limb. Calculations were performed on data from both the anterior and posterior projections. Furthermore, geometric mean, that is, the square root of the product of anterior and posterior counts, was calculated.

Scintigraphy was performed for 1 min to obtain a benchmark. For exsanguination, the right leg was elevated to 60° for 1 min followed by inflation of a 14-cm wide pneumatic cuff mounted on the thigh to a pressure of 300 mmHg. The limb was realigned and a 1-min scintigram was obtained. The tourniquet was deflated for 12 min to eliminate the effect of hyperaemia before the next exsanguination. The percentage reduction of blood volume was then calculated from counts obtained before and after the exsanguination, respectively. Because of the short time between these measurements, counts were not corrected for physical decay of 99mTc.

Lower limbs – reproducibility

In order to assess the reproducibility of measurement performed after the combined exsanguination and alignment procedures, the procedures and measurements were repeated seven times in four of the subjects. To evaluate the reproducibility of the alignment procedure alone, the limb was removed and realigned in the frame through six series of measurements with exsanguination maintained by keeping the tourniquet inflated.

Lower limbs – myoglobin

Recordings of serum myoglobin were made before and after the experiments in all 12 subjects having the thigh tourniquet.

Fig. 1. Each subject was placed supine with the crura aligned in a frame on the Gamma camera in order to reduce variation of positioning.
Ten healthy male volunteers with a mean age of 26 years (range 20–34) had autologue injection of 99mTc-radiolabelled erythrocytes with a mean radioactivity of 797 MBq (range 694–888). The subjects were placed supine with the right hand and arm on a single-headed gamma camera (StarCam XR/7, Star 4000i, GE Medical Systems, Wisconsin, Ill, USA). Tape markings were used for correct alignment of the arm on the gamma camera (Fig. 2). To achieve a reproducible proximal positioning of a ROI for subsequent integration of radioactivity, a 57Co source was placed 3 cm distal to the medial epicondyle. As ROI we used the smallest rectangle that included the right hand and the part of the right forearm distal to the marking. Scintigraphy was performed for 1 min to obtain a benchmark. For exsanguination the right arm was elevated to vertical for 30 sec followed by inflation of the 14-cm wide pneumatic cuff mounted on the upper part of the arm to 200 mmHg. The arm was realigned and a 1-min scintigram was obtained. The tourniquet was deflated for 5 min to eliminate the effect of hyperaemia before the next exsanguination. The fractional reduction of blood volume was then calculated from counts obtained before and after the exsanguination, respectively. Comparison of scintigraphic and plethysmographic figures included adjustments for radioactive decay.

The experiments assessing reproducibility were performed in four subjects according to the principles described for the lower limb.

In addition to the scintigraphic measurements, we performed water-plethysmographic measurements on upper limbs using a commercially available volumeter (Forearm Volumeter, Volumeters Unlimited, Phoenix, USA). Water-plethysmographic measurements were done in every subject before and after the exsanguination caused by elevation of the upper limb for 30 sec. Furthermore, the volumetric measurements were performed in relation to the seven repeated scintigraphic measurements.

The local committee of ethics for Copenhagen approved the study, which was carried out in accordance with the recommendations of the Declaration of Helsinki.

In order to analyse the distribution of data, we used the Shapiro-Wilks W-test and a normal probability plot. One-way analysis of variance (ANOVA test) for repeated measurements was applied to compare mean values of results obtained from anterior projection, posterior projection and geometric mean. The variance in results obtained during repeated combination of exsanguination and alignment procedures was compared with the variance in results obtained during repeated alignment procedures alone by means of F-test for repeated measurements. The coefficient of variation was defined as the standard deviation as a percentage of the mean. Linear regression analysis was used to compare scintigraphic and volumetric data. Serum levels of myoglobin before and after the experiments were compared by means of a paired t-test. A p-value <0.05 was considered significant in all tests.

All data were normally distributed. After 5 min of rest after 1-min use of the tourniquet, the following values in fractions of the benchmark values were found: in the lower limbs experiment 1.001 ± 0.045 (2 SD) and correspondingly in the arm experiment 0.982 ± 0.073 (2 SD).
Lower limbs and upper limbs – procedure

An example of scintigrams obtained from the lower limb experiment in one subject and the upper limb experiment in another is shown in Fig. 3.

Lower limbs – scintigraphic projection

In fractions, the mean value for one measurement of reduction of blood content in 12 subjects was $0.49 \pm 0.14$ (2 SD) for the anterior projections, $0.47 \pm 0.16$ (2 SD) for the posterior projections and $0.48 \pm 0.15$ (2 SD) for geometric means. On comparing these three mean values, no significant difference was found. Referring to the anterior projection the coefficient of variation between subjects was 14%.

Lower limbs – reproducibility

Referring to fractions, the SD obtained during repeated combinations of exsanguination and alignment procedures was 0.028 and when compared with the SD of 0.025 obtained during repeated alignment alone, no significant difference was found. The coefficient of variation within subjects was 6% after the combined exsanguination and alignment procedures. No change was seen in the results obtained through the experiment with the repeated alignments, i.e. there was no ooze of blood under the cuff. Results obtained from the four subjects in the reproducibility experiment are shown in Fig. 4.

Lower limbs – myoglobin

Mean concentration of serum myoglobin was 2.2 nmol/l (range 1.5–5.9) before the experiment and 1.9 nmol/l (range 1.5–2.6) after it. The difference was not significant.

Upper limbs – scintigraphic projection

In fractions, the mean value for one measurement of percentage reduction of blood content in 12 subjects was $0.45 \pm 0.10$ (2 SD). The

Fig. 3. Example of scintigrams obtained in lower and upper limb experiments before and after exsanguination caused by elevation.

Fig. 4. Results obtained from the four subjects in respectively the upper and lower limb reproducibility trials and showing the fractional reduction in blood volume. The error bars represent 1.96 SD.
between-subjects coefficient of variation was 11%.

**Upper limbs – reproducibility**

SD obtained during repeated combination of exsanguination and alignment procedures was 0.028, and when compared with the SD of 0.023 obtained during repeated alignment alone, no significant difference was found. The within-subjects coefficient of variation was 6% after the combined exsanguination and alignment procedures. No change was seen in the results obtained through the experiment with the repeated alignments, i.e. there was no ooze of blood under the cuff. Results obtained from the four subjects in the reproducibility experiment are shown in Fig. 4.

**Upper limbs – plethysmography**

The median and quartile value of reduction of the forearm was 27.6 ml (12.7–62.1), as measured by the volumetric method. The between-subjects coefficient of variation was 57% and, correspondingly, the within-subject variation was 53%. A significant relation was found between the volumetric and the scintigraphic results (see Fig. 5) ($r = 0.42$, $p = 0.018$).

**DISCUSSION**

A heterogeneous distribution of blood in the lower limb causing a non-uniform attenuation of gamma radiation could have influenced our results. However, the geometric mean corrections did not show any impact of the results. For practical proposes, therefore, we recommend the use of only one projection.

In the lower limb experiment the mean reduction in blood volume caused by elevation for 1 min was 0.49 using the anterior projection and, correspondingly, the mean reduction in blood volume in the upper limbs caused by the elevation for 30 sec was 0.45. Only future studies can clarify whether these figures are high or low compared to exsanguinations caused by other procedures.

The increase in blood volume caused by hyperaemia after deflation of the tourniquet had for all practical purposes normalized in 5 min ($1.001 \pm 0.045$ [2 SD]); we therefore reduced the pause between exsanguination to only 5 min in the upper limb experiment.

In order to determine whether the within-subject variation of blood volume reduction was physiologic or technical, we performed the reproducibility experiment. We did not find any difference in reproducibility between the

![Fig. 5](image-url)  
Fig. 5. Relation between scintigraphic and plethysmographic measurements in the upper limbs experiment. The stippled line represents the 95% confidence limits, and the full-drawn line the regression line ($r = 0.42$, $p = 0.018$).
experiment with the six combined exsanguination and realignment procedures and the six replacements alone in either the lower or the upper limb experiment. This meant that the majority of the within-subject variation was caused by technical limitations, and we believe that the inaccuracy of the measurements should be referred mainly to the alignment of the limb and the placement of the marker. In future studies using our method, it will therefore be important that care is taken with alignment of the limb and with placing the marker at precisely the same spot every time. The within-subject coefficient of variation was 6% in the lower limb experiment and 6% in the upper limb experiment; these figures are low compared to other studies dealing with limb volumes [10, 11, 14, 19, 23, 24].

We chose to compare our scintigraphic method with the water plethysmographic method as this method, in contrast to the strain gauge method, offers the opportunity to evaluate reductions of local blood volume caused by external methods of exsanguinations such as Esmarchs bandages.

The strain gauge plethysmographic method was used by Manyari et al. [17], who, during pressure volume determinations, found a correlation between the gauge estimates of increasing volume change and the scintigraphic estimate. Correspondingly, we found a significant correlation between the results of decreasing volumes obtained by the water-plethysmographic method and the scintigraphic results. However, we believe that the scintigraphic method is superior to any plethysmographic method regarding determinations of changes in blood volumes in limbs, because the scintigraphic method is more precise and represents a more physiologic approach. The plethysmographic method is an indirect method in which limb volume is measured and not blood volume. The scintigraphic method is more direct when measuring labelled erythrocytes, although some of them might diffuse extravascularly. In reference to exsanguination, we hypothesize that the fractional reduction is mainly a decrease in the venous blood volume, while the decreased blood volume in the arterial system and capillaries is limited. Physiologically speaking, we believe that some of the interstitial fluid is drained when a limb is elevated for more than a few seconds. Though we cannot estimate the quantitative error this could cause, it might be of some importance in relation to minutes of elevation, as can be seen before orthopaedic surgery. Warren et al. [12] found a maximum exsanguination after 5 min of elevation using a strain gauge plethysmographic method, but their conclusion may be false because of the drainage of interstitial fluid. Contrary to previous scintigraphic methods [17, 19], by clamping the residual blood volume we have been able measure a momentary volume of blood very precisely by means of 1 min of scintigraphy. Comparing this value with a benchmark, we have been able to determine the relative change, which has made it possible to measure the amount of blood volume reduction. Our method expresses blood volume changes as a percentage, and cannot yield quantitative results of volume changes, e.g. ml blood.

The blood flow in the limbs was effectively stopped, since no rise in counts was seen during the experiment with repeated realignments, though it lasted about 10 min. Though repetitive, only transitory tourniquet applications were done, and we observed no rise in serum myoglobin. This is contrary to reports from Ikomoto et al. and Jorgensen et al., who found some rise in serum myoglobin in relation to surgery [25, 26]. This indicates that the thigh muscle trauma is of minor importance using 1-min repetitive tourniquet inflations to 300 mmHg.

The topic of limb blood volumes is of importance when studying venous physiology. We believe that the method presented here can be used when investigating physiologic and pathophysiologic mechanisms in relation to the blood volumes of limbs that have not been open to research previously.

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