ASSSESSMENT OF THE RANGE OF NORMAL VALUES OF THE LOWER LIMB PERFUSION INDICATORS AT REST AND STRESS WITH THE OWN RADIO ISOTOPIC METHOD AND PROGRAM

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ABSTRACT

Lack of muscle perfusion normal values limited the hitherto diagnostics to the comparative evaluation of relative perfusion without the possibility to define the range of norm. Muscle perfusion, being a physiological process should have quantitative parameters defining the range of normal values. Nuclear medicine is vested with possibilities, methods and markers that allow us defining muscle perfusion. We took on the trial to elaborate the method and program that solve this issue in a very useful way for clinical diagnostics.

Aim: Elaborating the method and program allowing for half-quantitative evaluation of the lower limbs’ perfusion at rest and stress, and defining the range of normal values of the lower limbs’ perfusion indicators at rest and stress allowing for their clinical application.

Material: As the criterion for inclusion in a group - was the lack of dysfunctions in the lower limbs’ vascular system. In all, 74 non-smoking persons (34 women and 40 men), age between 21 and 45 (average age 31.8) were examined.

Methods: I. Examinations that excluded the circulation impairment in the lower limbs’ muscles were: basic tests, biochemical blood tests, USG-Doppler, ankle-brachial indicator.

II. Radio isotopic examinations at rest and stress were performed with the gamma camera ELSCINT SP 6HR with high-resolution collimator 99mTc-99mMIBI with the 11.1 MBq/kg m.c. activity. The results were mathematically analysed with the own program ALLP (Assessment Lower Limbs Perfusion). The results of basic tests and USG-Doppler didn’t show any deviation from the norm. The elaborated radio isotopic method and program allowed for defining the range of normal values of calves’ perfusion, which is contained in the range 5.28–8.02 at rest and 4.23–6.32 at stress; in the case of thighs’ perfusion the range of normal values was: 4.54–7.08 at rest and 4.00–5.66 at stress.

Conclusions: 1. The elaborated radioisotopic method and program allow for half-quantitative assessment of the lower limb perfusion at rest and stress.

2. Radio isotopic determination of the range of normal values of muscle perfusion at rest and stress allows for differentiating the patients with normal and impaired lower limb circulation.

3. Established normal ranges of the lower limb perfusion values at rest and stress allow for their clinical application both in the diagnostics of healthy patients and in the diagnostics of dysfunctions of the muscle perfusion. They also assist in the monitoring of effects in the conservative and operative treatment and other factors and drugs that influence the muscle perfusion.

Key words: Scintigraphy, muscle perfusion, rest, stress, perfusion indicator, normal values, lower limbs.

INTRODUCTION

Lack of muscle perfusion normal values limited the hitherto diagnostics to the comparative evaluation of relative perfusion without the possibility to define the range of norm. Being a physiological process, the muscle perfusion should have quantitative parameters that define the range of normal values.

SAŽETAK


Cilj: 1. Izraditi metod i program koji omogućuje polu-kvantitativnu proce nu perfuzije donjih udova u stanju mirovanja i opterećenja. 2. Definisati opseg normalnih vrednosti indikatora perfuzije donjih udova u stanju mirovanja i opterećenja koji omogućava njihovu kliničku primenu.

Materijal: Kriterijum za uključivanje u grupu je bilo odsustvo disfunkcija u vaskularnom sistemu donjih udova. Ispitane su 74 osobe, nepušača (24 žene i 40 muškaraca) starije dobi između 21 i 45 godina (prosječ na starost 31,8).

Metod: Ispitivanje kliničkih grupa je isključivalo oštećenje cirkulacije u mišićima donjih udova: osnovni testovi, biohemijički testovi krvi, USG-Dopler, brahio-maleolarni indeks. Radioizotopska ispitivanja u stanju mirovanja i opterećenja su izvedena uz pomoć gamma kamere ELSCINT SP 6HR sa kolimatorom 5-HR visoke rezolucije nakon ubrizgavanja radiofarmaceutskog agens 99mTc-99mMIBI uz 11,1 MBq/kg m.c. aktivnosti. Rezultati su matematički analizirani kroz vlastiti program PPDU (Procena Perfuzije Donjih Udova). Rezultati osnovnih testova i USG-Doplera nisu pokazali odstupanje od norme. Izrađeni radioizotopski metod i program omogućava definisanje opsega normalnih vrednosti perfuzije mišića, ona se nalaze u opsegu 5,28–8,02 u stanju mirovanja i 4,23–6,32 u stanju opterećenja, u slučaju perfuzije nakanulena, opseg normalnih vrednosti je 4,54–7,08 u stanju mirovanja i 4,00–5,66 u stanju opterećenja.

Zaključak: 1. Izrađen radioizotopski metod i program omogućava polu-kvantitativnu procenu perfuzije donjih udova u stanju mirovanja i opterećenja. 2. Radioizotopska određivanje opsega normalnih vrednosti perfuzije mišića u stanju mirovanja i opterećenja omogućava razlikovanje pacijenata sa normalnom i oštećenom cirkulacijom donjih udova. 3. Ustvarjanje normalnega opsega vrednosti perfuzije donjih udova v stanju mirovanja in opterećenja omogoča njihovo klinično primeno v dijagnostiki zdravih pacijentov in v dijagnostiki disfunkcije perfuzije mišića. Oni takože pomažu v kontroli efekta konservativnega in operativnega lečenja kot in drugih faktorov in lekov, ki utiče na perfuzijo mišića.

Ključne reči: Scintigrafija, perfuzija mišića, stanje mirovanja in opterečenja, indikator perfuzije, normalne vrednosti, donji udovi.

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The lower limbs’ perfusion impairment, caused by numerous disturbances, is especially at initial stages of the metabolic and circulatory system diseases, hardly noticeable both for the patient and doctor (1,2). However, lack of clear clinical symptoms doesn’t mean that there are no existing regional disturbances in the circulatory system (3). Stress also clearly influences the change in the muscle perfusion. In the pathological conditions, perfusion may highly differ from normal values, which is dictated by the level of endothelium impairment or vessel impairment. Even more important and calling for further investigations is the phenomenon of changed endothelium reaction to stress observed in the number of metabolic and circulatory system diseases (4).

These pieces of information create the necessity for searching of the method that would allow, in non-invasive way, for defining the normal range of muscle perfusion, that would help in determining the deviations from the normal state of perfusion and at the end contribute to early recognition of the stage of development of this pathology at different stages of the disease. Nuclear medicine is equipped with possibilities, methods and markers that allow for defining the blood perfusion in muscles. Accumulation of the given isotopic marker allowing us to define the muscle perfusion may depend on impaired vascular blood flow, dysfunctions of its transfer through vascular wall and impaired metabolic activity of muscle cells.


Further works concerning this matter, allowing us to take into consideration the elements important for the blood perfusion such as the whole body area of the examined patient are necessary. Also defining the upper and lower limit of normal values contributed to the modification of computer program and even more precise definition of the range of normal values of the lower limb perfusion of a healthy person.

The aims of our study were:
1) Elaboration of the method and program allowing for half-quantitative assessment of the lower limbs’ perfusion at rest and stress.
2) Establishing the range of values of indicators of the normal lower limb perfusion at rest and stress allowing for their clinical application.

MATERIAL AND METHODS

The perfusion was assessed by testing the subjects in whom, during the objective and subjective clinical examinations and in the USG and laboratory tests we didn’t find any circulation dysfunctions in the lower limbs’ vascular system. The material consisted of 74 non-smoking subjects, age 21–45, (average age 31,8), 34 women and 40 men directed to radio-isotopic perfusion screenings of the lower limbs at rest and stress. The inclusion criteria were: normal weight (18 ÷ 25 BMI), lack of dysfunctions of circulation in the lower limbs’ vascular system defined on the basis of anamnesis and general, clinical and laboratory researches.

The protocol of the researches was accepted by The Ethics Committee of the Medical University in Łódz.

Methods

I. Clinical, graphic and laboratory researches confirming the inclusion criteria and excluding the dysfunctions of circulation in the lower limbs’ vascular system.

1) Group of initial researches:
   - age, sex, height, weight, systolic and diastolic blood pressure and ankle-brachial indicator (Wk-r – the range of indicator normal values 0,9–1,2) (6).
   - general and biochemical blood tests: morphology, glucose, cholesterol, triglycerides, and hepatic tests.

2) USG researches – all subjects underwent the USG researches with Doppler effect and Colour and Power Doppler. Those researches were carried out with the ACUSON 128 XP/10 and 3,5 MHz and 7 MHz sond. The left and right side of the patient’s body were examined. In carotid IMT CCA was defined. In back tibial and dorsal arteries V max, V min, TAMX, PI, RI, S/D were estimated.

II. Radio-isotopic researches

Radio-isotopic researches at rest and stress were carried out with the gamma camera ELSCINT SP-6HR with collimator of high resolution 5-HR. The researches were carried out at rest and stress. The acquisitions were taken 5 minutes after the injection of radiopharmaceutical agent Tc 99mMIBI with 11,1 MBq/kg m.c. activity. The exercise test was carried out on cycle ergometer BTL. The standard protocol of load was used. The load was increased every 3 minutes by 25 watts. In the exercise test the injection of indicator was executed after the achievement of sub maximal effort.

In order to indicate the lower limbs’ perfusion, the acquisition of the whole body in back projection was performed with the recorder standard position and 30 cm/min pass speed. Indeed, the whole body scintigram allows for isolating the area of thighs and calves’ muscles for the mathematical analysis and calculating perfusion indicators, however, with the relatively fast recorder pass, the calculations statistics wasn’t sufficient and that’s why, in the elaborated method, static acquisitions of thighs and calves were performed in the back projection in 300 seconds. To place the subject in a symmetrical way, own sub program allowing for proper placement of the subject in relation to recorder was used. This is a very important element in proper calculation of the established perfusion parameters.

The obtained figures and information within them underwent the mathematical analysis with the new program ALLP (Assessment Limb Lower Perfusion). To avoid the operator error, the process of analysis was maximally automated. The analysis of the obtained pictures began with putting in the one image the whole body scintigram in the 512x256 matrix. Next, the image was smoothed.
with 9-point filter and cleaned with the CLEAR function. On such prepared ROI describing the whole body was generated, and the information within it was closely bound with the body area of the examined subject. ROI was automatically generated with the isolation of the foil „from below” at the level of 2 points on 255 scale, which is only ~0.8% of the whole information. In the case of clear focus in the place of injection, alternatively, ROI was generated to decrease the calculated value by this part of the activity, which did not join the bloodstream.

Despite the fact that cleaning and filtering of the image undoubtedly causes the loss of minimal part of information, it is however essential for the automatic generation of ROI describing the whole body. The filtering of the image was performed automatically - thus the occurring error was stable throughout the research. From the ROI describing the whole body the number of calculations per one pixel of matrix was calculated.

Next the area of thighs and the upper calves’ muscles was analysed. In every case the images were filtered with 9-point filter and cleared with the CLEAR function. Just like in the case of the whole body scintigram, this process was to prepare the images for the automatic generation of the areas of interest (ROI) describing the calves and thighs’ muscles. Such prepared image was analysed in terms of indicating the max point of generation, in which the profilogram was drawn separately for calves and thighs’ muscles. From the obtained profilogram data, the level of the foil isolation 20% from above and 10% from below was established and ROI describing the given muscle group was generated. The designated ROI was copied as a mirror image into the second limb. In analogy with the case of the whole body scintigram, during the analysis of the images of calves and thighs, the minimal part of initial information was lost as a result of processing. However, with total automation of the process, the occurred error was stable for each patient, the conclusion was drawn that it could be omitted in further calculations.

Each examination at rest and stress was elaborated separately. The ROIs of thighs and calves’ muscles were analysed in terms of the total number of calculations and number of calculations per one pixel of matrix. The value of general calculations allowed for calculation of factors of perfusion symmetry from the formula:

\[
WS = \frac{\text{ROI with the fewer number of calculations}}{\text{ROI with the greater number of calculations}} \times 100\%
\]

to avoid the situation in which the symmetry factor (WS) could be greater than 100.

The proper ROIs were analysed in terms of calculated parameters, and always in the numerator there was ROI with fewer number of calculations. The result presented in the report alternatively showed whether it is a relation of the left side to the right or the other way round.

Defining the symmetry rates of the lower limbs’ perfusion was not the aim of this study, that’s why while analysing the group of healthy subjects this issue was omitted in further elaboration of the research results. However, they are very useful and important in the case of circulation disturbances in different diseases.

The number of calculations per one pixel of matrix (calculations density) in given muscles and the number of calculations per one pixel of matrix (calculations density) in the whole body allowed for the calculation of factors of perfusion (WP) - from the formula:

\[
WP = 1 - \frac{\text{density count in select ROI}}{\text{density count in the whole body}} \times 100
\]

To estimate recurrence of the method, the phantom was used on which the described research was carried out. Knowing the activity in given areas, calculations were performed with the elaborated program ALLP. They were in accordance with the taken assumptions. And recurrence of the program was checked by numerous studies of the same research. The obtained results differed only in one thousandth of the calculated parameters and proved the proper algorithm of the studied program.

### III Statistical methods.

The resulting data were analysed with the „Statistica 7.1’’ program - to determine, whether the obtained results of radio isotopic research have normal distribution. Following the average, median standard; maximum and minimum deviation were calculated.

In designation of the new range of normal values we used the rule that the norm are those values that are in the range:

\[
\{\text{value}_{\text{average}} \pm 2\text{SD}\}
\]

### RESULTS

Research excluding (excluded) the possibility of dysfunctions in the circulatory system of lower limbs.

Results of the laboratory research presented in the Table 1 didn’t show any deviations from the norm that could influence the circulatory system.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average</th>
<th>Standard deviation</th>
<th>Value range</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>31.80</td>
<td>7.68</td>
<td>21 ÷ 45</td>
</tr>
<tr>
<td>weight (kg)</td>
<td>62.70</td>
<td>9.56</td>
<td>44 ÷ 79</td>
</tr>
<tr>
<td>height (cm)</td>
<td>162.01</td>
<td>6.21</td>
<td>146 ÷ 181</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122.43</td>
<td>8.79</td>
<td>115 ÷ 150</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73.15</td>
<td>86.1</td>
<td>70 ÷ 90</td>
</tr>
<tr>
<td>RBC (x10^6/ml)</td>
<td>4.73</td>
<td>0.53</td>
<td>3.33 ÷ 5.10</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>15.21</td>
<td>1.05</td>
<td>13.80 ÷ 17.60</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>46.81</td>
<td>3.88</td>
<td>40 ÷ 51</td>
</tr>
<tr>
<td>WBC (x10^3/ml)</td>
<td>5.98</td>
<td>1.11</td>
<td>4.40 ÷ 8.10</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>0.98</td>
<td>0.09</td>
<td>0.80 ÷ 1.10</td>
</tr>
<tr>
<td>GLU (mg/dl)</td>
<td>83.15</td>
<td>12.10</td>
<td>65 ÷ 108</td>
</tr>
<tr>
<td>CHOL (mg/dl)</td>
<td>5.24</td>
<td>0.26</td>
<td>4.81 ÷ 6.12</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.01</td>
<td>0.11</td>
<td>0.94 ÷ 1.26</td>
</tr>
<tr>
<td>AspAT</td>
<td>27.34</td>
<td>6.23</td>
<td>18 ÷ 37</td>
</tr>
<tr>
<td>ALAT</td>
<td>21.11</td>
<td>4.97</td>
<td>7 ÷ 32</td>
</tr>
</tbody>
</table>
The results of ankle-brachial indicator (W k-r) were contained in the range 09÷1,20 (average value 1,08; standard deviation 0,05) and didn’t show any deviations from the norm. In all patients the results of USG Doppler didn’t show any important chemodynamic disturbances in the blood flow of the lower limbs’ vessels. Also the measured parameters of neck vessels didn’t differ from normal values.

**Results of radio-isotopic studies**

The results are presented in Table 2 and in figures 1

**Table 2.** Calculated values of perfusion indicators (WP).

<table>
<thead>
<tr>
<th></th>
<th>Perfusion indicator at rest</th>
<th>Perfusion indicator after effort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>podudzia WPP</td>
<td>uda WPU</td>
</tr>
<tr>
<td>Average value</td>
<td>6,61</td>
<td>5,81</td>
</tr>
<tr>
<td>median</td>
<td>6,62</td>
<td>5,86</td>
</tr>
<tr>
<td>Max value</td>
<td>6,93</td>
<td>5,96</td>
</tr>
<tr>
<td>Min. value</td>
<td>5,82</td>
<td>4,91</td>
</tr>
<tr>
<td>Standard deviation SD</td>
<td>0,68</td>
<td>0,63</td>
</tr>
<tr>
<td>Upper limit of normal values</td>
<td>8,02</td>
<td>7,08</td>
</tr>
<tr>
<td>Lower limit of normal values</td>
<td>5,28</td>
<td>4,54</td>
</tr>
</tbody>
</table>

![Figure 1. Normal result of the lower limbs' perfusion study at rest - report.](image)

**DISCUSSION**

The diseases impairing the circulation and the lower limbs’ perfusion are important medical and economic problem. The costs caused by them are very high (7,8,9). The progress and increasing interest in researches on pathogenesis of disturbances in the lower limbs’ perfusion are observed. Those actions especially include new diagnostic techniques and cheap methods (10–20). The use of Tc99m MIBI in the research of the lower limbs’ perfusion emerges from particular qualities of the molecule of the compound. Highly selective transfer to the inside of the muscle cell depends on the proper work of the cell’s energetic mechanisms. In dysfunctions of these mechanisms a decrease of membrane potential and quick washout of the muscle cell indicator are observed (21). In relation to this, in the area of perfusion dysfunctions, we should expect a lesser uptake of indicator in the pathology affected muscle groups and individual muscles (22).

Changed behaviour of the lower limbs’ muscle perfusion at stress can be explained by changed behaviour of vascular endothelium which plays an important role in the keeping up the circulatory system haemostasis, controls blood flow, regulates the plates’ activity, coagulation cascade and fibrinolysis and is responsible for the proper tissue hydration (23,24). Stress also causes higher isolation of endogenic nitric oxide in reaction to the greater vascular flow speed (25). It also takes an active part in the regulation of the vascular flow through the production of ERDF factor (15,26).

Different methods were used to examine the lower limbs’ perfusion, yet each of them has some deficiencies or limits (17,27). Thus, USG Doppler doesn’t give a full view of the system of small vessels of the circulatory system (6,13,28,29). Classical angiography carries the risk connected with giving the contrast medium and exposure to ionising radiation; the latest digital angiography and NMR angiography don’t cause these limits, yet, they bring little to the case of tissular perfusion in the area of supply of a given vessel (1,30,31). This method also doesn’t give the possibility to compare the lower limb perfusion at rest and stress. None of these methods is as closely tied to the energetic metabolism of muscle cell as perfusion scintigraphy performed with the Tc99m MIBI.

That is why the technique that is used in diagnostics of the lower limbs’ perfusion is perfusion scintigraphy performed with Tal T1 201or much cheaper Tc99m MIBI (4,20,32,33,34,35,36,37,38). There are not many reports concerning this issue and there was no defined range of normal values of the lower limbs’ perfusion both at rest and stress (5,35,36,37).

In our researches, as a reference value, we took the indicator generation by muscles of the whole body in the back projection, as muscle groups in the back of the body in normal conditions are developed proportionally to the lower limbs’ muscles and seldom undergo vascular changes, which makes them the best reference value. Some of the authors take other muscle groups (e.g. forearm muscle group) as the reference point. As we’ve highlighted above, values obtained in the exercise tests that were 10 % higher should be explained by normal endothelium reaction to exercise, the influence of adenosine, being the vasodilatating factor and the eruption of EDREF factor – nitric oxide that causes the vasodilatation (39,40,41).

The choice of cycle ergometer to the stress tests has its grounds in equal load of particular muscle groups. It loads mainly muscles of the lower legs, which causes that such indicator, as heart action (sub maximal exercise) is the best for equal load for all subjects.
We assumed that the examined group of subjects didn’t have any circulatory disturbances in the lower limbs. This allowed for taking the assumption that the obtained results give the basis to adopt them as normal value, contained in the range calculated from the formula:

\[
WPP = \frac{\text{average } WPLP + \text{average } WPPP}{2} \pm 2SD
\]

WPP – calves’ perfusion indicator
WPLP – left calf perfusion indicator
WPPP – right calf perfusion indicator

On the basis of results we defined: the range of normal values of calves’ perfusion comprises in the range 5,80 ± 6,83 at rest, and 4,91 ± 5,63 after exercise.

For normal thighs’ muscle perfusion indicators we took the values contained in the range calculated from the formula:

\[
WPU = \frac{\text{average } WPLU + \text{average } WPPU}{2} \pm 2SD
\]

WPU – thighs’ indicator
WPLU – left thigh indicator
WPPU – right thigh indicator

The range of normal values of thigh perfusion comprises in the range 4,93 ± 5,75 at rest, and 4,35 ± 5,01 after exercise.

The obtained indicators allow for defining the normal lower limbs’ perfusion and make possible the diagnostics and monitoring of dysfunctions of this circulation. The modified in terms of mathematical analysis of the picture our own program analysing the calculations density in the examined muscle groups is deprived of the averaging elements and allows for precise half-quantitative analysis in the precisely defined areas of interest, conveying the particular lower limbs’ muscles groups. Also in the research of influence by various drugs on the muscle perfusion.

**REFERENCES**