STEREOLOGICAL ANALYSIS OF THE HUMAN PONS VASCULAR NETWORK

Zdenka Krivokuća, Vesna Gajanin*, Tatjana Bucma*, Slađana Ugrenović**, Dejan Zdravković** and Ivan Jovanović***

Stereological analysis of the human pontine vascular network was carried out on 10 human ponses. Multipurpose test system M-42 according to Weibel was used on the light microscope, with 40x lens magnification and with conventional stereological procedures. We determined the following stereological parameters: volume density (Vv), surface density (Sv), length density (Lv), and absolute number of blood vessels on the pontine field of vision (N). V_v=0,0423±0,0011053 (mm^0), S_v=4,275±0,1257554 (mirr'), lv=44,4601±4,2412984 (mm-2), and N is 5,771±0,7675277. We compared these results with the stereological measuring results of vascular network in: caudate nucleus, lentiform nucleus, thalamus, precentral gyrus and substantia nigra.

Pons is supplied by blood vessels of considerable caliber, according to the established volume density of higher values, and lower absolute number and surface density values than the same parameters in the compared structures. *Acta Medica Medianae 2003; 42 {2): 17-21.*

Key words: *pons, vascular network, stereology*

Department of anatomy, Medical faculty, Banja Luka*,
Department of anatomy, Medical Faculty, Nis**

Correspondence to: Zdenka Krivokuća
Department of anatomy of Medical faculty, 78000 Banja Luka
Bosnia and Herzegovina

Introduction

Blood vessels which supply pons are the branches of the basilar artery, anterior inferior cerebellar artery and superior cerebellar artery (1, 2). These branches for pons can be divided into anterior (ventral), lateral and posterior (dorsal) branches. The anterior group of vessels are the branches of basilar artery whereas the lateral group presents the branches of basilar artery, anterior inferior cerebellar artery (AICA) and superior cerebellar artery (SCA). The dorsal group of vessels are the branches of SCA (2, 3).

Anterior pontine arteries end in the basilar sulcus regions. This sulcus is supplied by these arteries as well (2, 4). The lateral group of arteries runs across the pyramidal eminence and come to the trigeminal nerve root, middle and superior cerebellar peduncles, which are supplied by these vessels (5, 6). Dorsal pontine arteries are less numerous and supply superior cerebellar pedunculus, mesencephalic tract of trigeminal nerve and locus ceruleus (7).

We have used the stereological methods to create the clear picture of blood vessels density, their distribution and area. Stereology is the branch of morphology which three-dimensionaly interprets flat areas and sections by geometrical probability. The basic tasks of stereology are to define three-dimensional shapes, volumes and volume relations, areas volume-area relations, the size of particles distribution, their number in the volume unit, and the length of twisted elements (8).

Aim

The aim of this work was to determine the basic stereological characteristics of the pontine vascular network.

Material and methods

The researches were conveyed on 10 brains of adultd corpses of both sex from 35 to 85 years old. Material was collected at Sremska Kamenica Institute fot lung diseases and at Department for pathology of Clinical Center Banja Luka.

Brains were processed by the usual autopsy technique, and were removed from the cranial cavity. The brain stems were separated from the leptomeningies and the rest of the brains and then fixed into the 10% formaldehyde.

All ponses were sectioned in three layers (upper, middle and lower) for the purpose of stereological analysis-stratified sampling. All layers were sectioned in transverse plain, and their thickness was 5 mm, anterior 4 mm below prepontine fossa, middle in the

level of the trigeminal nerve root, and posterior 4 mm above postpontine fossa. Semiserial sections (5, 10, 15, 20. cut) were made from these samples. Visualisation of blood vessels was performed with histochemistry method for blood vessels according to Mallory (9) (Fig. 1). Control of the regular blood vessels verification was performed by immunohistochemistry method with antigene factor VII (10) (Fig. 2). Two ponses were immunohistochemistry stained with this method, for testing the precision of our blood vessel counting. We compared results from sections with different staining and statistically processed with t-test foraverage values equality.

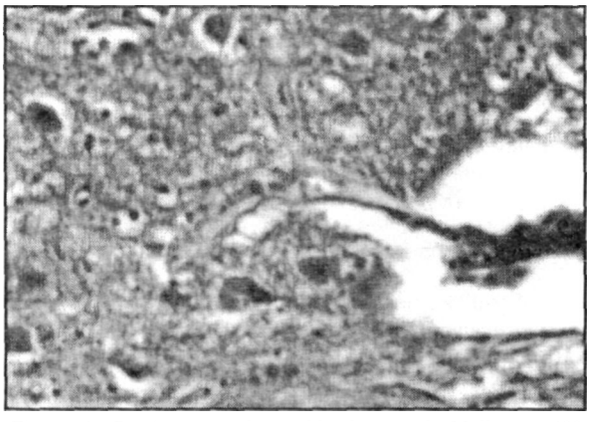

Figure 1. Pontine parenchyma blood vessel, Mallory x 400

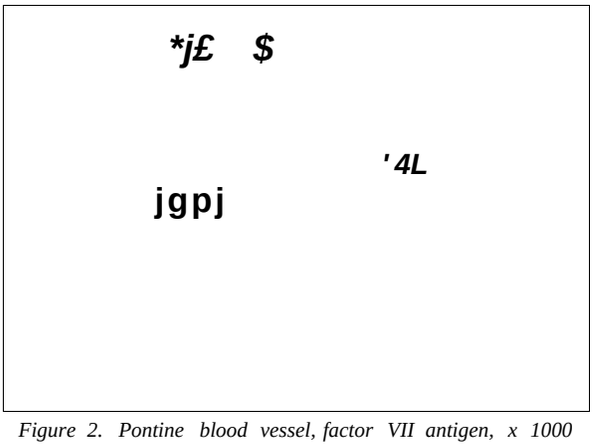

Figure 2. Pontine blood vessel, factor VII antigen, x 1000

Multipurpouse test system M-42 according to Weibel (11), built in the eyepiece, with 40x lens magnification, of the "Opton" light microscope. The selection of the samples (fields of vision) was performed systematically-intermittently, every second field of vision, and the size of the sample, or necessary the number of stereological measuring for every variable and every group was performed according to De Hoff formula (12).

This measuring was carried out on 120 paraffin pontine sections. The measuring of volume density was carried out on 972 test fields, surface density on 972 test fields, length density on 1968 test fields as well as the absolute number of blood vessels on 288 test fields.

Stereological analysis included relative stereological variables of pontine blood vessels (arteries): volume density (Vv), surface density (Sv) and lenght density (Lv) according to Weibel, with conventional stereological procedures usage (11).

The results were presented tubular, such as mean values (X), standard deviation (S) and standard error (SE).

Table 1. Pontine vascular network volume density values

Case number	$V_{V(SS)}$ (mm^0)	$V_{V(SM)}$ (mm^0)	$V_{V(SI)}$ (mm^0)	V_v for whole pons (mm^0)
1.	0,052	0,034	0,039	0,042
2.	0,044	0,041	0,039	0,041
3.	0,036	0,036	0,034	0,035
4.	0,042	0,038	0,038	0,039
5.	0,037	0,043	0,043	0,041
6.	0,039	0,048	0,045	0,044
7.	0,043	0,044	0,037	0,041
8.	0,041	0,045	0,046	0,044
9.	0,053	0,047	0,055	0,051
10.	0,044	0,045	0,045	0,045

Results

The following parameteres were established by stereological analysis:

- Volume density of pontine vascular network.
- Surface density of pontine vascular network.
- Lenght density of pontine vascular network.
- Absolute number of blood vessels on the field of vision.

Volume density of pontine vascular network

Pontine vascular network volume density values par layer are presented in table 1 for the whole pons.

Mean value, standard deviation, standard error of pontine vascular network volume density are presented in table 2.

Table 2. Mean value, standard deviation and standard error of pontine vascular network volume density

	$V_{V(SS)}$ (mm^0)	$V_{V(SM)}$ (mm^0)	$V_{V(SI)}$ (mm^0)	Vvfor whole pons (mm^0)
X	0,0431	0,0421	0,0421	0,0423
S	0,00504	0,0018165	0,0029664	0,0034928
Se	0,0015949	0,0005748	0,0009387	0,0011053

Average volume density of pontine vascular network is:

V_v=0,0423±0,0011053 (mm^0)

Average volume density per layer (superior, middle and inferior stratum) is:

V_{vss}=0,043110,0015949 (mm^0)
V_{VSM}=0,0421 ±0,0005748 (mm^0)
V_{vSI}=0,0421±0,0009387 (mm^0)

Surface density of pontine vascular network

Surface density of pontine vascular network, values per layer (superior stratum, middle stratum and inferior stratum), for the whole ponse are presented in table 3.

Table 3. Pontine vascular network surface density values

Case number	^{SV}(SS) (mm^{-1})	^{SV}(SM) (mm^{-1})	^{Sv}(s.> (mm^{-1})	Sv for whole pons (mm^{-1})
1.	5,33	4,19	3,81	4,44
2.	3,94	4,75	3,71	4,11
3.	3,81	4,06	3,63	3,83
4.	3,68	3,68	3,81	3,71
5.	3,94	4,57	4,44	4,31
6.	3,81	5,33	4,19	4,44
7.	4,13	4,37	3,88	4,13
8.	4,13	4,44	4	4,19
9.	5,31	4,98	5,23	5,15
10.	4,24	7,72	4,37	4,44

Mean value, standard deviation and standard error of pontine vascular network surface density ar in the table 4.

Table 4. Mean value, standard deviation, standard error of pontine vascular network surface density

	^{SV}(SS) (mm^{-1})	^{SV}(SM) (mm^{-1})	SV$_{(SI)}$ (ram^{-1})	Sv for the whole pons (ram^{-1})
X	4,232	4,509	4,107	4,275
S	0,5981508	0,473391	0,4798854	0,3973872
Se	0,1892882	0,1498071	0,1518624	0,1257554

Average surface density of pontine vascular network is:

Sv=4,275±0,1257554 (mm^{-1})

Average surface density of pontine vascular network, per layer are:

S_{vss}=4,232±0,1892882 (mm^{-1})
S_{VSM}=4,509±0,1498071 (mm^{-1})
S_{vSI}=4,107±0,1518624 (mm^{-1})

Length density of pontine vascular network

Length density of pontine vascular per layer (superior stratum, middle stratum and inferior stratum) for whole pons are presented in table 5. Mean value, standard deviation and standard error of pontine vascular network length density values presented in table 6.

Table 5. Pontine vascular network length density values

Case number	$^{1\,V}$(SS) (mm^{-2})	^{LV}(SM) (mm^{-2})	I v ^{V}(SI) (mm^{-2})	Lvfor whole pons (mm^{-2})
1.	72,41	58,32	65,36	65,36
2.	34,05	45,40	41,49	40,31
3.	28,57	28,18	27	27,79
4.	36	28,18	30,14	31,31
5.	40,704	34,05	32,48	35,62
6.	36,39	34,05	38,36	36,007
7.	50,88	36,79	34,44	40,704
8.	52,05	50,49	48,53	50,09
9.	64,19	69,08	66,147	66,14
10.	51,27	50,49	52,05	51,27

Table 6. Mean value, standard deviation, standard error of pontine vascular network length density

	^{LV}(SS) (mm^{-2})	^{LV}(SM) (mm^{-2})	1 v ^{L}(SI) (mm^{-2})	Lv for whole pons (mm^{-2})
X	46,6514	43,503	43,599	44,4601
S	14,060646	13,615073	14,136001	13,402503
Se	4,4495715	4,3085674	4,473418	4,2412984

Average value of pontine vascular network lenght density is:

L_v=44,4601 ±4,2412984 (mm^{-2})

Average pontine vascular network length density, per layer values are:

Lv_{ss}=46,6514±4,4495715 (mm^{-2})
Lv_{SM}=43,503±4,3085674 (mm^{-2})
Lv_{SI}=43,599±4,473418 (mm^{-2})

Absolute pontine number of blood vessels par field of vision (N) is 5,771 ±0,7675277.

Results were compareed by t-test mean value equality statistical method, with the results on the same section, but with different staining (Mallory and antigen VII). We concluded that there is not statisticaly significant difference between these results. Therefore, we decided to use Mallory staining, mostly because of economic reasons.

Discussion and conclusion

There are very short data about pontine vascularisation in the recent literature. Present informations are related to obvious pontine blood vessel morphology, without stereological analysis of pontine vascular network. Therefore, we compared these results with the same parameters in other structures of the brain

such as lentiform nucleus, caudate nucleus, precentral gyrus, thalamus and substantia nigra (13, 14,15, 16).

Mallory staining was used during the stereological analysis. During the usage of this staining, not only the lumen of blood vessels was filled, but the structural components of blood vessels wall were stained too. The surface and volume density were defined not only for the lumen, but for the wall of blood vessels too. The influence of arteriosclerosis and degenerative changes of the intimal surface of blood vessels, or the influence of age was avoided.

We concluded that the vascular network of thalamus and substantia nigra are different from the vascular network of pons, according to the literature data. Pontine vascular network is larger than the thalamus and substantia nigra network. Pontine vascular network is somewhat larger than lentiform nucleus vascular network. Caudate nucleus volume density values (14) are the closest to the pontine vascular network values. There is hypothesis that the blood flow is slower in the larger vascular network, which gives the basis for the frequency of infarctions explanation, in the investigated structures (17, 18, 19, 20, 21, 22).

We compared mean values of pontine vascular network volume density between different layers, and we concluded that there was not any essential difference between different layer values. This suggests that the pontine vascular network is the similar in different pontine regions.

Surface density of thalamus and substantia nigra, are more similar to pontine vascular network surface density than lentiform nucleus, precentral gyrus and caudate nucleus values. There wasn't any essentially difference between values of the surface density of different pontine vascular network layers.

There is a large difference between the pontine vascular network and thalamus length density. Pontine vascular network values are higher than thalamus length densita values.

Average values of length densita in superior stratum are somewhat higher than in middle and inferior layer.

Absolute number of pontine blood vessels is smaller than the same lentiform nucleus, precentral gyrus and caudate nucleus values.

Pons is supplied by the blood vessels with larger caliber, according to the results of the stereology parameters, such as higher volume density values, smaller absolute number of blood vessels and lower surface density, than the same parameters in compared structures (caudate nucleus, lentiform nucleus, precentral gyrus, thalamus and substantia nigra).

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STEREOLOSKA ANALIZA VASKULARNE MREZE PONS-A ČOVJEKA

Zdenka Krivokuc'a, Vesna Gajanin, Tatjana Bućma, Slađana Ugrenović, Dejan Zdravković i Ivan Jovanović

Stereološka analiza vaskularne mreže ponsa je rađena na deset moždanih mostova. Korišten je višenamjenski testni sistem M-42 po *Weibel-u* na svjetlosnom mikroskopu i objektiv povecanja 40x uz konvencionalne stereoloske procedure. Određivani su sledeći stereoloski parametri: volumenska gustina (Vv), povrsinska gustina (Sv), dužinska gustina (Lv) i apsolutan broj krvnih sudova po vidnom polju (N) ponsa. V_v=0,0423±0,0011053 (mm^0), S_v=4,275±0,1257554 (mm-'), L_v=44,4601±4,2412984 (mm^{-2}), a N iznosi 5,771+0,7675277. Dobivene rezultate smo poredili sa rezultatima stereoloških mjerenja vaskularne mreže: nucleus (nc.) caudatus-a, nc. lentiformis-a, thalamus-a, gyrus precentralis-a i substantiae nigrae.

Moždani most vaskularizuju krvni sudovi velikog kalibra, s obzirom na ustanovljene visoke vrijednosti volumenske gustine, a znatno manji apsolutan broj krvnih sudova, i niske vrijednosti površinske gustine, u odnosu na istoimene vrijednosti poređenih struktura. *Acta Medica Medianae 2003; 42 (2): 17-21.*

Ključne reči: pons, vaskularna mreia, stereologija