УДК 616.831

LEUKOCYTES AS MARKERS OF HEMORRHAGIC STROKE INFLAMMATORY NATURE

A. N. Yabluchanskiy
University College London, United Kingdom

Hemorrhagic stroke in response to intracerebral hemorrhage is considered an aseptic form of inflammation. With this in mind the dynamics of leukocytes changes in blood and in the hemorrhagic stroke zone as main factors of inflammation are examined. Sprague-Dawley rats (N = 24) underwent surgical procedures and were divided into two groups — control group and a hemorrhagic stroke group — an injection of 2 μL of sterile saline and 2 μL of 0,2U of Collagenase-IV (S) respectively. White blood cells count analysis, combined histological and immunohistological examination with further densitometric and statistical analysis of blood and hemorrhagic stroke zone leukocytes were performed. A synchronized increase in blood and hemorrhagic stroke zone levels of both polymorphonuclear and mononuclear leukocytes was observed, highlighting the inflammatory nature of hemorrhagic stroke. A delay of 48 hours between peak levels of polymorphonuclear and mononuclear leuokocytosis both in blood and the hemorrhagic stroke zone was established. Therefore, understanding hemorrhagic stroke as one of the forms of aseptic inflammation suggests alternate perspectives in the development of new approaches to diagnosis and treatment including interventions into kinetics of blood and hemorrhagic stroke zone leukocytes.

KEY WORDS: hemorrhagic stroke, inflammation, collagenase-induced hemorrhagic stroke, experimental model, leukocytes, neutrophils, polymorphonuclears, mononuclears, micrgoglia, macrophages

ЛЕЙКОЦИТИ ЯК МАРКЕРИ ЗАПАЛЬНОЇ ПРИРОДИ ГЕМОРАГІЧНОГО ІНСУЛЬТУ

А. М. Яблучанський
Университетський коледж Лондона, Великобританія

Геморагічний інсульт, що розвивається у відповідь на інтрацерабральнІ геморагії, розглядається з позицій асептичної форми запалення. Досліджена динаміка змін лейкоцитів в загальній крові і безпосередньо зоні мозкового крововиливу. Експериментальні тварини, пацюки Сраг-Даул, (N = 24) після хірургічного втручання були розділені на дві групи — контролю та геморагічного інсульту після внутрішньомозкового — введення 2 мкл стерильного фізіологічного розчину або 2 мкл колагенази-IV (S) (0,2U), відповідно. Здійснені загальний аналіз крові, гістологічний та імуногістохімічний аналізи з подальшими денситометричним та статистичним методами оцінки. Спостерігалась синхронізація в підвищенні рівня поліморфноядерних і мононуклеарних лейкоцитів крові та зони пошкодження. Встановлено 48-годинне зміщення піків в рівнях поліморфноядерних і мононуклеарних лейкоцитів в загальному аналізі крові і зоні геморагічного інсульту. Таким чином, слід розглядати геморагічний інсульт в якості однієї з форм асептичного запалення, що може сприяти розвитку альтернативних підходів у діагностиці та лікуванні даного захворювання, в тому числі через втручання в кінетику лейкоцитів на загальному та місцевому рівнях.

КЛЮЧОВІ СЛОВА: геморагічний інсульт, запалення, колагеназа-індукований геморагічний інсульт, експериментальна модель, лейкоцити, нейтрофіли, поліморфноядерні клітини, мононуклеари, мікроглія, макрофаги

ЛЕЙКОЦИТЫ КАК МАРКЕРЫ ВОСПАЛИТЕЛЬНОЙ ПРИРОДЫ ГЕМОРРАГИЧЕСКОГО ИНСУЛЬТА

А. Н. Яблучанский
Университетский колледж Лондона, Великобритания

Геморрагический инсульт, развивающийся в ответ на интрацерабральные геморрагии, рассматривается в качестве асептической формы воспаления. Были исследованы динамика изменения лейкоцитов в общей крови и непосредственно в зоне геморрагического кровоизлияния. Экспериментальные животные, крысы Сраг-Даули, (N = 24) подверглись хирургическому вмешательству и были разделены на две группы — группу контроля и группу геморрагического инсульта — введение 2 мкл стерильного физиологического раствора или 2 мкл коллагеназы-IV(S) (0,2U), соответственно. Были осуществлены общий анализ крови, гистологические и иммуногистохимические анализы с последующими денситометрическими и статистическими методами оценки. Наблюдалась синхронизация в повышении уровня полиморфноядерных и мононуклеарных лейкоцитов. Также было установлено 48-часовое смещение пиков в уровнях полиморфноядерных и мононуклеарных лейкоцитов в общем анализе крови и в зоне геморрагического инсульта. Таким образом, следует рассматривать геморрагический инсульт в качестве одной из форм асептического воспаления, что может способствовать развитию альтернативных подходов в диагностике и лечении данного заболевания, включая и вмешательство в кинетику лейкоцитов на общем и местном уровнях.

КЛЮЧЕВЫЕ СЛОВА: геморрагический инсульт, воспаление, коллагеназа-индуцированный геморрагический инсульт, экспериментальная модель, лекоциты, нейтрофилы, полиморфноядерные клетки, мононуклеары, микроглия, макрофаги

INTRODUCTION

Stroke is the second main cause of death worldwide and a major reason of mortality and morbidity [1–3]. Hemorrhagic stroke, a result of rupture to a blood vessel in the brain, is poorly understood and relatively understudied [4–6].

Hemorrhagic stroke (HS) is accountable for 20 percent of stroke cases [7, 8]. 30 percent of patients die within the first month and 23 percent die after a year. Only 65 percent of survivors function independently [9–11], the rest are significantly disabled and may need considerable help with daily tasks [12–15].

The main goal for developing a treatment for hemorrhagic stroke patients lays in the fundamental understanding of the nature of its reparative mechanisms.

There are currently few *in vivo* models for studying hemorrhagic stroke, the most frequently used are: blood and collagenase injection into the brain of experimental animal [16–19]. In our study we gave the preference to the collagenase injection model based on its properties to destroy the basal lamina of the blood vessels, thus causing a «natural» blood leak into surrounding tissues, in contrast to blood injection when the blood is administered in bolus and does not represent the mechanistic nature of hemorrhagic stroke. The standardization of the damage in hemorrhagic stroke is of the upmost importance. The blood injection model has been reported to be inconsistent [17, 20], whilst the collagenase injection model seems to result in reproducible round-shape hematoma.

Hemorrhagic stroke represents one of the aseptic forms of inflammation as a compensatory defensive reaction of the organism to intracerebral hemorrhage damage. However, this concept of hemorrhagic stroke has not been developed further than a basic idea of inflammatory involvement. Present works that investigate certain markers of inflammatory reaction [2, 21–25] do not answer the problem as a whole, as they are directed to particular tasks and lay out the concept of inflammation as one of the natural reparative mechanism of hemorrhagic stroke.

One of the leading factors in the inflammatory process are leukocytes [26]. Polymorphonuclear leukocytes migrate to the lesion focus, «clean up» from the damaged tissues with further mononuclear reaction, which morphology is determined by the blood stem cells, and further connective tissue scarring [27]. The quality of these processes and the connective tissue scar as the result of these processes define the outcome of the inflammation [28–31]. Mononuclear leukocytes in the hemorrhagic stroke zone are represented by the microglial/macrophages reaction [32–35].

We have not found any publication that simultaneously examined changes in the blood leukocytes and in the hemorrhagic stroke zone hence the reasoning behind this current work.

MATERIALS AND METHODS

Animals and experimental design

24 Sprague-Dawley male rats (250–350 g) were divided into 2 groups: group of hemorrhagic stroke and control group with 12 animals in each respectively. Experimental animals were anesthetized with izoflurane. The midline incision was made in the dorsal region of the scalp, revealing sagital suture with location of bregma. Using stereotaxic frame (Stoelting) the cranial burr hole was drilled 3 mm lateral to bregma and 0,2 mm anterior. Using 26g needle 0,2U/2 µl Collagenase IV-S (Sigma Aldrich) or 2 µl of sterile saline were injected into the right basal ganglia (5,5 mm ventral from the skull surface). Infusion was performed over 2 minutes and the needle was kept at the place of injection for 2 minutes to avoid backflow of the fluid [18, 36]. Bone wax (Johnson and Johnson) was applied into the thickness of the bone and the wound have been closed with mirsilk sutures (Ethicon). Buprenorphine was used as an analgesic (0,1U) post operation subcutaneously. The model provided a development of intracerebral hemorrhage of standard size and localization (Fig. 1).

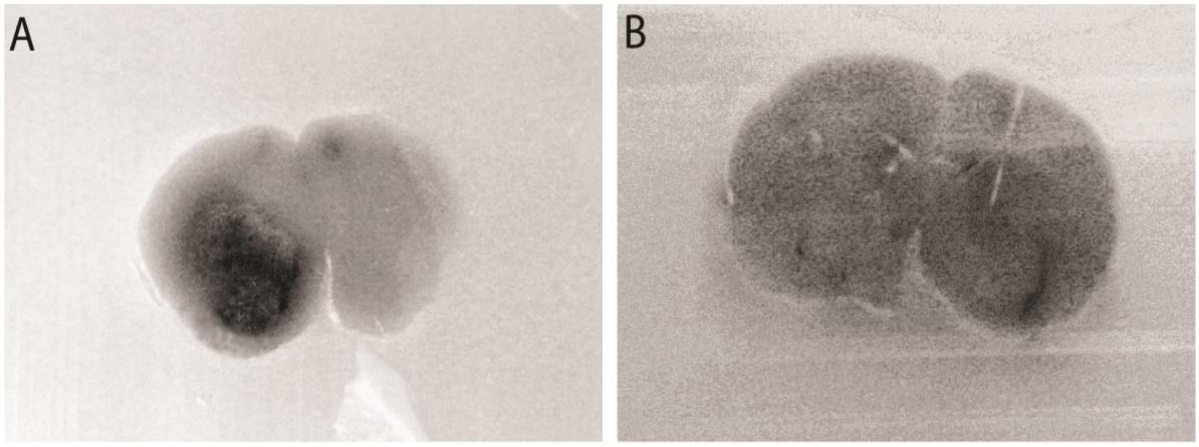

Fig. 1. Coronal section of the experimental rats brains performed through zone of injection of 0,2 U/2 µl collagenase IV-S (A) and sterile saline (B) on the first day post operation

Experimental animals were examined on days 1, 3, 5, 7, 14 and 21 post operation for white blood cells analysis, combined histological and immunohistological tests with subsequent densitometric analysis of polymorphonuclear leukocytes and microglial/macrophages.

The rats were sacrificed with lethobarb injection (1 ml i/p) according the procedures described in the Experimental Animals Act.

White blood cell analysis

Rat tail vein blood was collected in EDTA tubes and used for white blood cell counts using an ADVIA 2120 hematology system (Bayer Health Care). The total level of white blood cells ($*10e^6/\mu L$) was evaluated and the number of polymorphonuclears and lymphocytes (%) was calculated.

Myeloperoxidase staining

Slides were treated with APTS solution and airdried for 24 hours. Sample sections were cut at a thickness of 5 µM and placed on APTS slides. Each sample was dried over the night at 60 ˚C. Slides were dewaxed following standard procedures using xylene, IMS in different concentrations and running tap water. Antigen retrieval was performed using a citrate buffer in a pre-heated water bath for the purpose of breaking the protein cross-links, therefore unmasking the antigens and epitopes in formalin-fixed and paraffin embedded tissue sections, thus enhancing the staining intensity of antibodies. After that sections were washed in PBS and allowed to cool. Liquid blocker pen was used to contour the tissue in the slides to prevent the leak of the liquids used. Goat serum was used to block a non-specific-binding in sections. Slides were incubated overnight at 4 ˚C with rabbit anti-human myeloperoxidase primary antibody (Dako UK Ltd) in 1:250 dilution. The next morning slides were washed with PBS, treated with H_2O_2 (Sigma) and washed with PBS again, the slides were then incubated with ImmPress Kit secondary antibody (Vector). Before sections were exposed to 3,3'-diaminobenzidine tetrahychloride (DAB, Research Genetics), they were washed in PBS. Sections were counterstained with hematoxylin [32], differentiated in acid-alcohol, washed with hydrochloric acid and then rinsed in running tap water. Slides were then dehydrated using different concentrations of IMS and stored in xylene until ready to mount.

OX-42 staining

Slides were treated with APTS solution and airdried for 24 hours. Sample sections were cut at a thickness of 5 μM and placed on APTS slides. Each sample was dried over the night at 60 ˚C. Slides were dewaxed with standard procedures using xylene, IMS in different concentrations and running tap water. Antigen retrieval was performed using a citrate buffer in a preheated water bath for the purpose of breaking the protein cross-links, which unmasks the antigens and epitopes in formalin-fixed and paraffin embedded tissue sections, thereby enhancing the staining intensity of antibodies. After that sections were washed in PBS and left to cool. Liquid blocker pen was used to contour the tissue on the slides to prevent the leakage of the liquids used. Normal horse serum was used to block non-specific-binding in sections. Slides were incubated overnight at 4 ˚C with mouse anti-rat CD11b (OX42 clone) primary antibody (AbD Serotec) in 1:100 dilution. Next morning slides were washed with PBS, treated with H_2O_2 (Sigma) and washed with PBS again. ImmPressKit secondary antibody (Vector) was applied with slides afterwards. Before sections were exposed to 3,3'-diaminobenzidine tetrahychloride (DAB, Research Genetics), they were washed in PBS. Slides were differentiated in acid-alcohol, washed with hydrochloric acid and then rinsed in running tap water. Slides were then dehydrated using different conenctrations of IMS and stored in xylene until ready to mount.

Densitometric cell count

Densitometric analysis is performed on the brain slices stained for MPO, OX42 and hematoxylin using free available software ImageJ. Slice images are taken using DPX controller software microscopically at 400 times optical magnification in 7 random different fields of view. The number of cells is calculated in all the fields of view and presented as a mean for the slice. MPO positive stained cells were identified by the positive immunological DAB staining and nuclei counterstaining at x400 magnification. OX-42 positive stained cells were identified by the positive immunological DAB staining at x400 magnification.

An example of positive staining for polymorphonuclears and microglia/macrophages is represented on the Fig. 2.

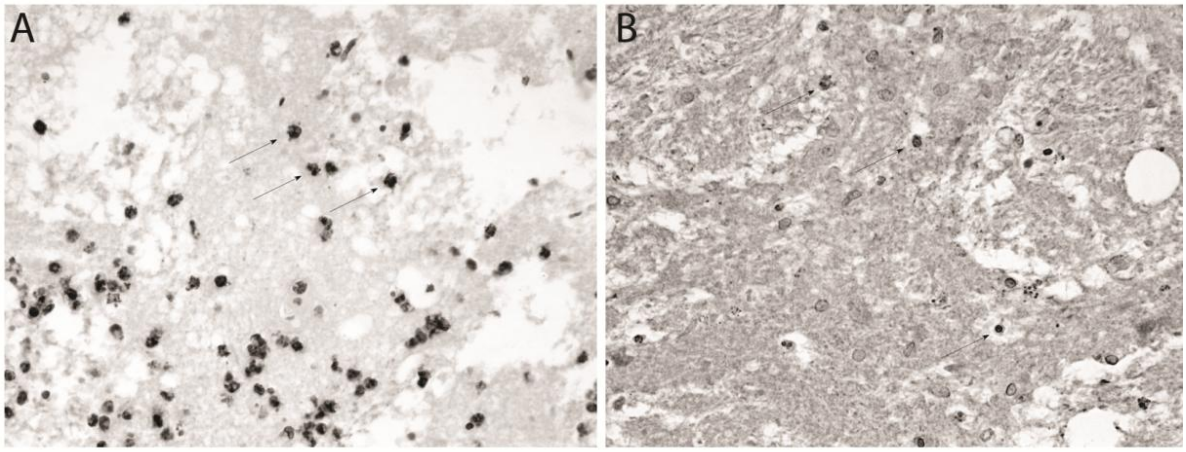

Fig. 2. Positive MPO stained polymorphonuclears, counterstained with hematoxylin (A). Positive OX-42 stained microglia cell/macrophage (B). X400 magnification

Statistical evaluation

Results obtained were statistically examined using SPSS Statistics 17.0 software. The mean and standard error of the mean were calculated. The significance of the differences between post-op data to pre-operational data in each animal group was evaluated using parametric (Student-test) and non-parametric (Mann-Whitney test). The data on the graphs is represented by the mean with standard error of the mean. Zero-point represents pre-operational data.

RESULTS

The saline injection group did not display any significant changes throughout the days of experiment. The collagenase injection group demonstrated a decrease in the number of white blood cells on day 1, however, the levels then increased to match those of the saline injection group by day 3. By day 7 the white blood cell count had again increased. Eventually by day 14 the WBC levels had fallen back to those of the saline. The data are represented on Fig. 3.

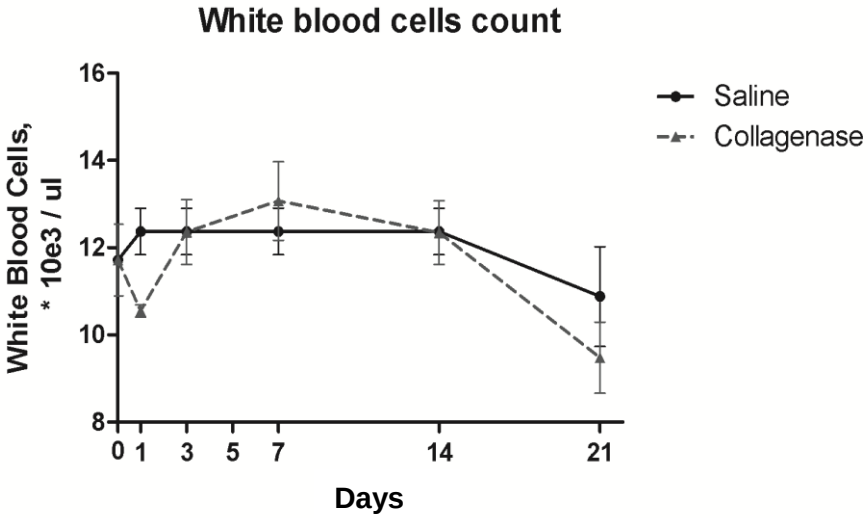

Fig. 3. White blood cells count over 21 days of experiment

The saline injection group showed a 5 % — increase on day 1 when compared to pre-operational data, which was lower than the one in collagenase injection group, with an eventual return to the state of pre-operational data by day 3 remaining at this level throughout the rest of experiment. Collagenase injection group showed rapid 10 % — increase in peripheral blood polymorphonuclears number on day 1, further rapid decrease on day 3, which was lower than pre-operational level by 5 %, with further 10 % — increase on day 7 and subsequent decrease and return to the state of pre-operational data by day 14 and 21. The data are represented in Fig. 4.

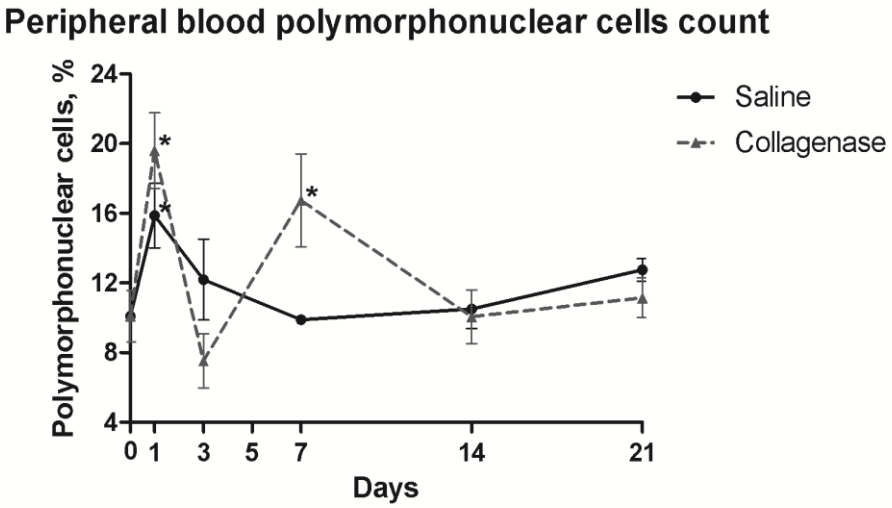

Fig. 4. Peripheral blood polymorphonuclear cells count over 21 days of experiment

The saline injection group indicated a small 2 % decrease on day 1, a 4 % increase on day 3, reaching the level of pre-operational data by day 7 with no further changes during the experiment. The collagenase injection group demonstrated a 6 % decrease in the number of peripheral blood lymphocytes on day 1, a pronounce and significant 12 % increase on day 3, with subsequent decrease by day 7, which was below pre-operational level. On the 14th day of the experiment there was a small increase in the number of peripheral blood lymphocytes in the collagenase injection group by 5 % followed by a subsequent decrease and reaching the pre-operational data by day 21. The data are represented on Fig. 5.

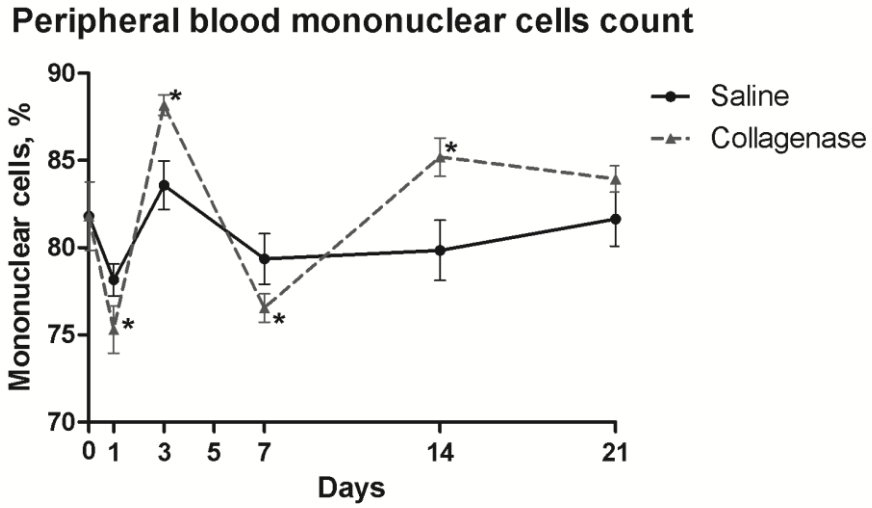

Fig. 5. Peripheral blood mononuclear cells count over 21 days of experiment

Densitometric cell count

No polymorphonuclears were present in the region of injection throughout the experiment in the saline injection group. This was in stark contrast to the collagenase injection group which demonstrated a significant 18-fold increase in the number of polymorphonuclears in the region of intracerebral hemorrhage on day 1. This increase, however, gradually decreased over days 3 and 5 reaching pre-operational data levels by day 7 and 14. The data are represented on Fig. 6.

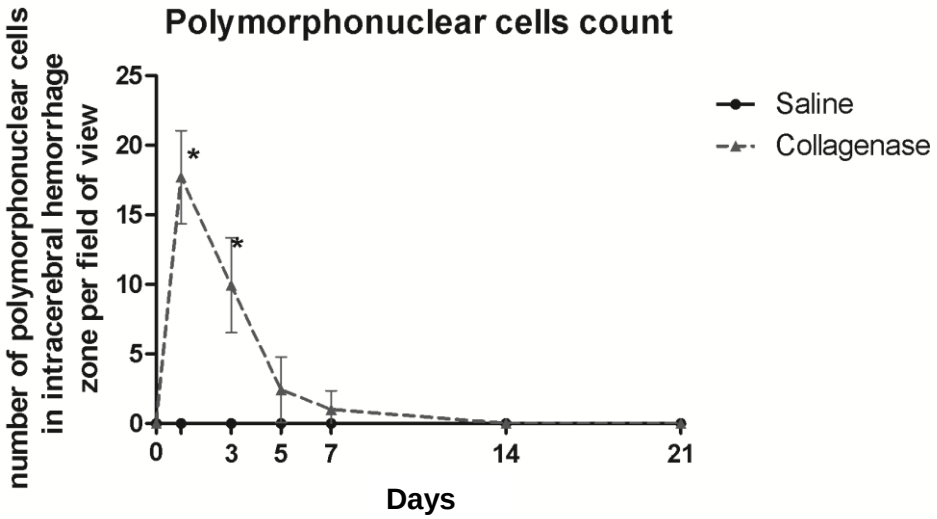

Fig. 6. Densitometric cell count analysis: number of polymorphonuclear cells in intracerebral hemorrhage zone per field of view

There was no difference in the number of microglial cells/macrophages compared to pre-operational data throughout the experiment in animals from the saline injection group. The collagenase injection group demonstrated a 20 — fold increase in the number of microglial cells/macrophages in the region of intracerebral hemorrhage on day 1, which was significantly higher than in saline injection group. By the 3rd day of experiment a further 1,3 — fold increase in the number of microglial cells/macrophages in collagenase injection group was observed. However, this was followed by a gradual 1,75 — fold decrease on day 5, reaching the level of the saline injection group and pre-operational data by days 7 and 14. The data are represented on Fig. 7.

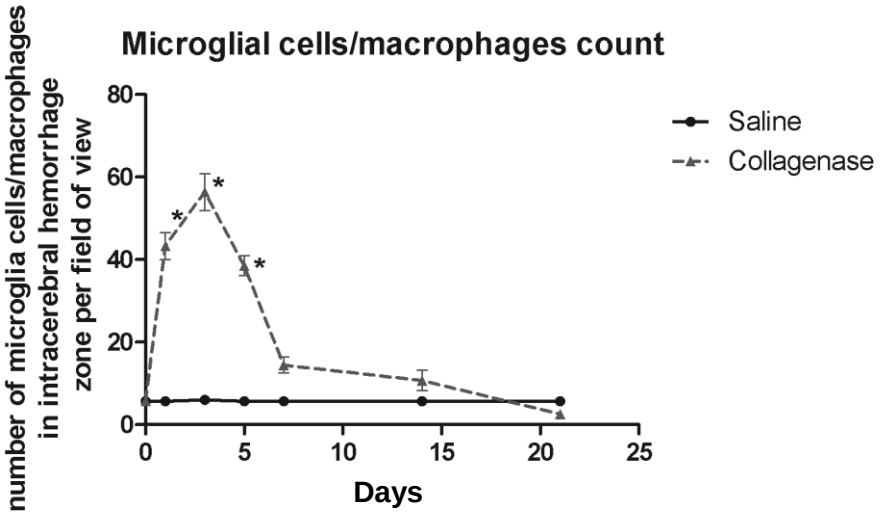

Fig. 7. Densitometric cell count analysis: number of microglia cells/macrophages in intracerebral hemorrhage zone per field of view

DISCUSSION

Our experiments determined leukocyte changes in the blood and in the zone of hemorrhagic stroke and correlate them with changes associated with aseptic inflammation of any nature.

System level of inflammation possesse a pivotal role in inflammatory response. It mobilizes organism system regulations as a result to injury and organizes a reparative process with the best possible outcome. The main role in the system level of inflammation belongs to the general adaptation syndrome or stress-syndrome [37].

One of the most important factors of system level of inflammation is leukocyte reactions in peripheral blood [38, 39]. These reaction form cell pools that generate leukocyte infiltrations in zones of damage, which provide destruction and elimination of injured structures and their replacement with connective tissue [44].

Most biochemical factors of inflammation, such as transaminases, phosphatases, reactive stage proteins, leukotrienes, cytokines (TNF alpha in particular) are connected with leukocyte reactions in peripheral blood [41–43].

Leukocyte reactions are generally greater in microbial inflammation and smaller in aseptic inflammation [44–46]. During microbial inflammation, the leukocytosis factors are represented by the products of microorganisms' catabolism and lesion focus, in contrast to aseptic inflammation when leukocytosis factors are represented by products of lesion focus only.

In our research, the saline injection group did not demonstrate changes in WBC from pre-operational data. The collagenase injection group demonstrated a decrease in the number of WBC on day 1 followed by an increase peaking on day 7 and subsequent reduction to the level of saline injection group and pre-operational data level. These changes of WBC in experimental animals subjected to collagenase injection meet a scheme of leukocyte changes in aseptic inflammation [47].

Leukocyte reaction during inflammation in experimental animals are mainly characterized with phase changes in quantity and activity of polymorphonuclears and lymphocytes [48–50], which is what we observed in the results of current paper.

By injecting saline, although there is no intracerebral bleeding, there is damage to the skull skin and bone integrity, to the brain tissue by needle insertion and pressurizing the tissue due to the liquid being injected, thus triggering an inflammatory response of the organism that was observed on a system level. This was demonstrated byan increase in peripheral blood polymorphonuclears on day 1 with further its stabilization by day 5 and a small increase in the number of peripheral blood lymphocytes on day 3 with further stabilization by day 5. Initial changes of leukocytes in the system level of saline injection group suggest adaptive mechanism of inflammation to any injury of the body, indicating an immediate response on the system level [25].

Polymorphonucleariс reaction in peripheral blood possesses phase characteristics. Although changes in peripheral blood polymorphonuclears in collagenase injection group are similar to the ones in saline injection group on day 1, the rate is much more pronounced. In contrast to the saline injection group, when starting from day 1 we observed a decrease in the number of peripheral blood polymorphonuclears towards the level of pre-operational data, the level of polymorphonuclears in the collagenase injec-

tion group demonstrated a significant decrease which was lower than in the pre-operational data on day 3 followed by a pronounced increase on day 7, which was stabilized to pre-operational data level only by day 14. A pronounced increase in the number of polymorphonuclears in peripheral blood on the first day of experimental hemorrhagic stroke may be explained with system reaction of the body to injury and stress and follows the processes that take place in aseptic inflammation [51]. A rapid decrease in the number of blood polymorphonuclears on day 3 in collagenase injection group can be explained by their consumption in zone of intracerebral hemorrhage, where the processes of destruction are under way [52], and by release of lymphocytes into the main stream [53]. Day 7 increase in the number of peripheral blood polymorphonuclears may be explained by large number of polymorphonuclears consumed with zone of intracerebral hemorrhage and thus the need to restore its numbers, and with their role in initiating the reparative processes [52].

Lymphocytic reaction in peripheral blood possesses phase characteristics as well. A rapid increase in their number on day 3 might develop from stimuli that come from a destruction zone and their decrease on day 5 might be explained by their consumption in zone of intracerebral hemorrhage. Further increased number of peripheral blood lymphocytes till the end of experiment correlates with patients' hemorrhagic stroke data and might be explained by change in their role from microglial cells/macrophages to the ones that provide a proliferative stage of inflammation of tissue replacement [54–57].

Changes observed in the number of blood leukocytes, phase changes of polymorphonuclears and lymphocytes in particular, in general correspond to the changes observed in human patients [24, 47, 58–62].

Being a physiological substance, saline injection did not result in any intracerebral hemorrhage in the hemisphere subjected to treatment. In contrast, collagenase injection resulted in a hematoma which occupied 30 % of the affected hemisphere. This is due to collagenase properties of disrupting a basal lamina of the vessels, thus contributing to the blood leak [63].

Saline injection did not affect the level of polymorphonuclears present in zone of needle insertion, nor any changes in the number of microglial cells/macrophages. In contrast, collagenase injection showed a rapid increase in the number of polymorphonuclears in zone of intracerebral hemorrhage, which peaked on day 1 and gradually decreased to the level of pre-operational data by day 5 and 7. As for microglial cells/macrophages, collagenase injection group showed an increase in their number in the region of intracerebral hemorrhage with maximal peak on day 3 with subsequent gradual decrease and reaching the levels of pre-operational data.

An increased number of polymorphonuclears in the zone of intracerebral hemorrhage correlated with other authors, who indicated polymorphonuclear infiltration in other experimental models of hemorrhagic stroke to begin and be present in the zone of intracerebral hemorrhage within 12 hours post ictus [32, 64]. Polymorphonuclear infiltration within the zone of intracerebral hemorrhage in our experimental model correlated with the basis of aseptic inflammation whereby polymorphonuclear attraction into zone of injury with the purpose to destroy damaged tissues [35], with other inflammatory models of hemorrhagic stroke [32, 40] and human patients' data with hemorrhagic stroke [65, 66]. The destruction of damaged tissue in early stages of inflammation occurs via a variety of bioactive enzymes released from polymorphonuclears in the zone of injury, such as gelatinase, collagenase, glucoronidase, elastase, myeloperoxidase, phosphatase, etc. [67, 68, 69, 70, 71]. Polymorphonuclears activity and attraction is regulated via production of a various cytokines [72, 2], e. g. TNF-alpha has been shown to mediate apoptosis and burst of polymorphonuclears [42], produced by macrophages and various antibodies produced in body to control polymorphonuclears life span [73]. Barone and Feuerstein showed that peripherally derived cytokines can cross the blood-brain barrier [74], the permeability of which increases after intracerebral hemorrhage [75]. There is a strong link between polymorphonuclears in the early stage of inflammation to its next stage of microglia/marophageall infiltration and this was described by Atzeni et al., who demonstrated that polymorphonuclears are responsible for early activation of T- and B-lymphocytes [76].

An increase in the number of microglial cells/macrophages that was observed in our experimental model of hemorrhagic stroke, which peaked on day 3, is explained by demands of lesion focus, that is already undergoing the processes of destruction, elimination of damaged tissue, that agrees with basis of aseptic inflammation. It was shown that microglial cells/macrophages are engaged with the number of cytokines, which in our case are widely produced by polymorphonuclears and with products of catabolism in the injured zone, thus gaining a signal for their further activation and infiltration [77, 2]. Microglial cell/macrophages

changes correlated with human patients data suffering hemorrhagic stroke [78, 46] and work by other authors in their experimental models of hemorrhagic stroke [64, 79, 80] which indicated them to peak around 48 hours post ictus. Finishing their cleavage role, microglial cells/macrophages undergo apoptosis as a preparation for the next stage of inflammation. Bzowka et al. demonstrated that macrophages undergoing apoptosis change their cytokine production profile, indicating a downregulation of pro-inflammatory cytokines and upregulation of anti-inflammatory ones, leading to proliferation stimulation [81]. Apart from cytokines produced in microglial cells/macrophages, a variety of proteases released from those, such as cathepsins for example, contribute to tissue repair as well [82].

Further decreases in the number of polymorphonuclears and microglial cells/ macrophages in the zone of intracerebral hemorrhage and their increase in peripheral blood that was observed on day 7 for polymorphonuclears and prolonged increase in the number of peripheral blood lymphocytes starting from day 14 suggest their involvement in the signalling and engagement process of recovery. Butterfield et al. demonstrated these cells to be responsible and strongly connected to the reparative processes running in inflammation [52]. A strong connection between cell kinetics in zone of intracerebral hemorrhage and in peripheral blood suggests a strong link between system level with local level of inflammation.

CONCLUSION

Hemorrhagic stroke should be considered one of the aseptic forms of inflammation as a response to intracerebral hemorrhage damage. The main role during these phenomena belongs to the leukocytes, which should be investigated in a combined study on the blood and hemorrhagic stroke zone levels. Dynamics of changes in blood leukocytes and leukocytes in the hemorrhagic stroke zone are synchronized by time. There is approx 48 hours delay in the peak levels between polymorphonuclear and mononuclear leuokocytosis both in blood and hemorrhagic stroke zone.

If we consider hemorrhagic stroke as one of the forms of aseptic inflammation we can develop new approaches to its diagnosis and treatment and consider interventions into kinetics of blood and hemorrhagic stroke zone leukocytes.

REFERENCE LIST

1. Frizzell J. P. Acute stroke : pathophysiology, diagnosis, and treatment / J. P. Frizzell // AACN.Clin.Issues. — 2005. — № 16 (4). — P. 421—440.
2. Wang J., Dore S. Inflammation after intracerebral hemorrhage / J. Wang, S. Dore // Journal of Cerebral Blood Flow and Metabolism. — 2007. — № 27 (5). — P. 894—908.
3. Worthley L. I., Holt A. W. Acute haemorrhagic stroke / L. I. Worthley, A. W. Holt // Crit Care Resusc. — 2000. — № 2 (3). — P. 209—219.
4. Badjatia N., Rosand J. Intracerebral hemorrhage / N. Badjatia, J. тр2цRosand // Neurologist. — 2005. — № 11 (6). — P. 311—324.
5. Rincon F, Mayer SA. Clinical review : Critical care management of spontaneous intracerebral hemorrhage. Crit Care. — 2008. — № 12 (6). — P. 237.
6. Smith EE, Rosand J, Greenberg SM. Hemorrhagic stroke. Neuroimaging Clin.N.Am. — 2005. — № 15 (2). — P. 259—72, ix.
7. Gere J. Epidemiology of hemorrhagic strokes / [J. Gere, D. Minier, G. V. Osseby, G. Couvreur, T. Moreau, F. Ricolfi, M. Giroud] // Journal of Neuroradiology. — 2003. — № 30 (5). — P. 291—297.
8. Testai F. D., Aiyagari V. Acute hemorrhagic stroke pathophysiology and medical interventions : blood pressure control, management of anticoagulant-associated brain hemorrhage and general management principles / F. D. Testai, V. Aiyagari // Neurol.Clin. — 2008. — № 26 (4). — P. 963—985.
9. Bamford J. A Prospective-Study of Acute Cerebrovascular-Disease in the Community - the Oxfordshire Community Stroke Project 1981-86.2. Incidence, Case Fatality Rates and Overall Outcome at One Year of Cerebral Infarction, Primary Intracerebral and Subarachnoid Hemorrhage / J. Bamford, P. Sandercock, M. Dennis, J. Burn, C. Warlow // Journal of Neurology Neurosurgery and Psychiatry. — 1990. — № 53 (1). — P. 16—22.
10. Lovelock C. E. Change in incidence and aetiology of intracerebral haemorrhage in Oxfordshire, UK, between 1981 and 2006 : a population-based study / C. E. Lovelock, A. J. Molyneux, P. M. Rothwell // Lancet Neurol. — 2007. — № 6 (6). — P. 487—493.
11. Santalucia P. Intracerebral hemorrhage : medical treatment / P. Santalucia // Neurol.Sci. — 2008. — № 29 Suppl 2. — S271—S273.
12. Guresir E. Subarachnoid hemorrhage and intracerebral hematoma : incidence, prognostic factors, and outcome / [E. Guresir, J. Beck, H. Vatter, M. Setzer, R. Gerlach, V. Seifert, A. Raabe] // Neurosurgery. — 2008. — № 63 (6). — P. 1088—1093.

13. Konatschnig T. Ten years' experience at a major stroke center / [T. Konatschnig, A. Knoll, A. Hug, W. Hacke, P. Ringleb] // Nervenarzt. — 2009. — № 80 (2). — P. 166—173.
14. Schepers V. P. Functional recovery differs between ischaemic and haemorrhagic stroke patients / V. P. Schepers, M. Ketelaar, A. J. Visser-Meily, J. W. de G. V. Twisk, E. Lindeman // J. Rehabil. Med. — 2008. — № 40 (6). — P. 487—489.
15. Wang D. Z., Talkad A. V. Treatment of intracerebral hemorrhage : what should we do now? / D. Z. Wang, A. V. Talkad // Curr.Neurol.Neurosci.Rep. — 2009. — № 9 (1). — P. 13—18.
16. He Y. Autophagy after experimental intracerebral hemorrhage / [Y. He, S. Wan, Y. Hua, R. F. Keep, G. Xi] // Journal of Cerebral Blood Flow and Metabolism. — 2008. — № 28 (5). — P. 897—905.
17. MacLellan C. L. Skilled reaching impairments follow intrastriatal hemorrhagic stroke in rats / C. L. MacLellan, S. Gyawali, F. Colbourne // Behavioural Brain Research. — 2006. — № 175 (1). — P. 82—89.
18. Strbian D. Rodent models of hemorrhagic stroke / D. Strbian, A. Durukan, T. Tatlisumak // Curr. Pharm. Des. — 2008. — № 14 (4). — P. 352—358.
19. Wasserman J. K. Glial responses, neuron death and lesion resolution after intracerebral hemorrhage in young vs. aged rats / J. K. Wasserman, H. Yang, L. C. Schlichter // European Journal of Neuroscience. — 2008. — № 28 (7). — P. 1316—1328.
20. Xue M. Comparison of brain cell death and inflammatory reaction in three models of intracerebral hemorrhage in adult rats / M. Xue, M. R. Del Bigio // J. Stroke Cerebrovasc. Dis. — 2003. — № 12 (3). — P. 152—159.
21. Bestue-Cardiel M. Leukocytes and primary intracerebral hemorrhage / [M. Bestue-Cardiel, J. Martin-Martinez, C. Iturriaga-Heras, J. R. ra-Callizo, A. Oliveros-Juste] // Rev. Neurol. — 1999. — № 29 (10). — P. 968—971.
22. Godoy-Torres D. A., Pineiro G. Inflammatory response in spontaneous intracerebral haemorrhage / [D. A. Godoy-Torres, G. Pineiro] // Rev.Neurol. — 2005. — № 40 (8). — P. 492—497.
23. Guo F. Q. Study of relationship between inflammatory response and apoptosis in perihematoma region in patients with intracerebral hemorrhage / F. Q. Guo, X. J. Li, L. Y. Chen, [et al.] // Zhongguo Wei Zhong. Bing. Ji. Jiu. Yi. Xue. — 2006. — № 18 (5). — P. 290—293.
24. Perera M. N. Inflammation following stroke / M. N. Perera, H. K. Ma, S. Arakawa, D. W. Howells, R. Markus, C. C. Rowe, G. A. Donnan // Journal of Clinical Neuroscience. — 2006. — № 13 (1). — P. 1—8.
25. Yoshimoto Y. Acute systemic inflammatory response syndrome in subarachnoid hemorrhage / Y. Yoshimoto, Y. Tanaka, K. Hoya // Stroke. — 2001. — № 32 (9). — P. 1989—1993.
26. Suzuki S. Acute leukocyte and temperature response in hypertensive intracerebral hemorrhage / [S. Suzuki, R. E. Kelley, B. K. Dandapani, Y. Reyes-Iglesias, W. D. Dietrich, R. C. Duncan] // Stroke. — 1995. — № 26 (6). — P. 1020—1023.
27. Andres R. H. Cell replacement therapy for intracerebral hemorrhage / [R. H. Andres, R. Guzman, A. D. Ducray, P. Mordasini, A. Gera, A. Barth, H. R. Widmer, G. K. Steinberg] // Neurosurg.Focus. — 2008. — № 24 (3–4). — E16.
28. Dziedzic T. Clinical significance of acute phase reaction in stroke patients / T. Dziedzic // Front Biosci. — 2008. — № 13. — P. 2922—2927.
29. Qu C. C. Diagnosis and treatment of chronic expanding intracerebral hematoma / [C. C. Qu, R. Yu, Z. G. Wang, S. J. Xu, G. M. Xu, Y. G. Liu, Q. L. Zhang] // Zhonghua Yi. Xue. Za Zhi. — 2008. — № 88 (11). — P. 746—748.
30. Silva Y. Molecular signatures of vascular injury are associated with early growth of intracerebral hemorrhage / [Y. Silva, R. Leira, J. Tejada, J. M. Lainez, J. Castillo, A. Davalos] // Stroke 2005. — № 36 (1). — P. 86—91.
31. Welsh P. Associations of proinflammatory cytokines with the risk of recurrent stroke / P. Welsh, G. D. Lowe, J. Chalmers, D. J. Campbell, A. Rumley, B. C. Neal, S. W. MacMahon, M. Woodward // Stroke 2008. — № 39 (8). — P. 2226—2230.
32. Gong C. Acute inflammatory reaction following experimental intracerebral hemorrhage in rat / C. Gong, J. T. Hoff, R. F. Keep // Brain Research. — 2000. — № 871 (1). — P. 57—65.
33. Hickenbottom S. L. Nuclear factor-kappaB and cell death after experimental intracerebral hemorrhage in rats / S. L. Hickenbottom, J. C. Grotta, R. Strong, L. A. Denner, J. Aronowski // Stroke 1999. — № 30 (11). — P. 2472—2477.
34. Wu J. Microglial activation and brain injury after intracerebral hemorrhage / J. Wu, S. Yang, G. Xi, S. Song, G. Fu, R. F. Keep, Y. Hua // Acta Neurochir.Suppl. — 2008. — № 105. — P. 59—65.
35. Xue M. Intracerebral injection of autologous whole blood in rats : time course of inflammation and cell death / M. Xue, M. R. Del Bigio // Neurosci. Lett. — 2000. — № 283 (3). — P. 230—232.
36. Rodrigues C. M. P. Tauroursodeoxycholic acid reduces apoptosis and protects against neurological injury after acute hemorrhagic stroke in rats / [C. M. P. Rodrigues, S. Sola, Z. H. Nan, R. E. Castro,

P. S. Ribeiro, W. C. Low, C. J. Steer] // Proc Natl Acad Sci U S A. — 2003. — № 100 (10). — P. 6087—6092.
37. Selye H., Fortier C. Adaptive reaction to stress / H. Selye, C. Fortier // Psychosom. Med. — 1950. — № 12 (3). — P. 149—157.
38. Becker K. J. Inflammation and acute stroke / K. J. Becker // Curr. Opin. Neurol. — 1998. — № 11 (1). — P. 45—49.
39. Kleinig T. J., Vink R. Suppression of inflammation in ischemic and hemorrhagic stroke : therapeutic options / T. J. Kleinig, R. Vink // Curr. Opin. Neurol. — 2009. — № 22 (3). — P. 294—301.
40. Frangogiannis N. G. Targeting the inflammatory response in healing myocardial infarcts / N. G. Frangogiannis // Curr.Med.Chem. — 2006. — № 13 (16). — P. 1877—1893.
41. Carlson N. G. IL-1 alpha, IL-1 beta, IL-6, and TNF-alpha impart neuroprotection to an excitotoxin through distinct pathways / N. G. Carlson, W. A. Wieggel, J. Chen, [et al.] // J.Immunol. — 1999. — № 163 (7). — P. 3963—3968.
42. Otsuka Y. Inhibition of neutrophil migration by tumor necrosis factor. Ex vivo and in vivo studies in comparison with in vitro effect / Y. Otsuka, K. Nagano, K. Nagano, K. Hori, J. Oh-ishi, H. Hayashi, N. Watanabe, Y. Niitsu // J.Immunol. — 1990. — № 145 (8). — P. 2639—2643.
43. Zaremba J., Losy J. Cytokines in clinical and experimental ischemic stroke / J. Zaremba, J. Losy // Neurol. Neurochir. Pol. —2004. — № 38 (1 Suppl 1). — P. S57—S62.
44. Emsley H. C., Tyrrell P. J. Inflammation and infection in clinical stroke / H. C. Emsley, P. J. Tyrrell // J. Cereb. Blood Flow Metab. — 2002. — № 22 (12). — P. 1399—1419.
45. Nakai K. Brain abscess following intracerebral haemorrhage / K. Nakai, T. Yamamoto, S. Yasuda, A. Matsumura // J. Clin. Neurosci. — 2006. — № 13 (10). — P. 1047—1051.
46. Wang J., Dore S. Inflammation after intracerebral hemorrhage / J. Wang, S. Dore // J. Cereb. Blood Flow Metab. — 2007. — № 27 (5). — P. 894—908.
47. Christensen H., Boysen G. C-reactive protein and white blood cell count increases in the first 24 hours after acute stroke / H. Christensen, G. Boysen // Cerebrovasc. Dis. — 2004. — № 18 (3). — P. 214—219.
48. Kitahara M. Changes in peripheral lymphocytes count in unconscious patients treated at home with Japanese herbal medicines (Hozai) / M. Kitahara, S. Ishikawa, K. Hara, S. Kanno, F. Ogata, K. Naruo, A. Yaguchi // Gan To Kagaku Ryoho. — 2002. — № 29 Suppl 3. — P. 526—529.
49. Rovlias A., Kotsou S. The blood leukocyte count and its prognostic significance in severe head injury / A. Rovlias, S. Kotsou // Surg. Neurol. — 2001. — № 55 (4). — P. 190—196.
50. Xue M., Del Bigio M. R. Intracerebral injection of autologous whole blood in rats : time course of inflammation and cell death / M. Xue, M. R. Del Bigio // Neurosci. Lett. — 2000. — № 283 (3). — P. 230—232.
51. Papassotiriou I. Severe aseptic inflammation caused by long distance running (246 km) does not increase procalcitonin / I. Papassotiriou, V. G. Alexiou, M. Tsironi, K. Skenderi, A. Spanos, M. E. Falagas // Eur. J. Clin. Invest. — 2008. — № 38 (4). — P. 276—279.
52. Butterfield T. A. The dual roles of neutrophils and macrophages in inflammation : a critical balance between tissue damage and repair / T. A. Butterfield, T. M. Best, M. A. Merrick // J.Athl.Train. — 2006. — № 41 (4). — P. 457—465.
53. Furze R. C., Rankin S. M. The role of the bone marrow in neutrophil clearance under homeostatic conditions in the mouse / R. C. Furze, S. M. Rankin // FASEB J. — 2008. — № 22 (9). — P. 3111—3119.
54. Boyce D. E. The role of lymphocytes in human dermal wound healing / D. E. Boyce, W. D. Jones, F. Ruge, K. G. Harding, K. Moore // British Journal of Dermatology. — 2000. — № 143 (1). — P. 59—65.
55. Farini A. T and B lymphocyte depletion has a marked effect on the fibrosis of dystrophic skeletal muscles in the scid/mdx mouse / A. Farini, M. Meregalli, M. Belicchi, M. Battistelli, [et al.] // J. Pathol. — 2007. — № 213 (2). — P. 229—238.
56. Huaux F. Eosinophils and T lymphocytes possess distinct roles in bleomycin-induced lung injury and fibrosis / [F. Huaux, T. Liu, B. McGarry, M. Ullenbruch, Z. Xing, S. H. Phan] // J. Immunol. — 2003. — № 171 (10). — P. 5470—5481.
57. Martin C. W., Muir I. F. K. The Role of Lymphocytes in Wound-Healing / C. W. Martin, I. F. K. Muir // British Journal of Plastic Surgery. — 1990. — № 43 (6). — P. 655—662.
58. Christensen H., Boysen G. C-reactive protein and white blood cell count increases in the first 24 hours after acute stroke / H. Christensen, G. Boysen // Cerebrovasc. Dis. — 2004. — № 18 (3). — P. 214—219.
59. Christensen H. Plasma cytokines in acute stroke / H. Christensen, G. Boysen, E. Christensen, H. H. Johannesen, K. Bendtzen // J. Stroke Cerebrovasc. Dis. — 2002. — № 11 (2). — P. 72—79.
60. Pedersen E. D. Systemic complement activation following human acute ischaemic stroke / E. D. Pedersen, U. Waje-Andreassen, C. A. Vedeler, G. Aamodt, T. E. Mollnes // Clin.Exp.Immunol. — 2004. — № 137 (1). — P. 117—122.

61. Suzuki S. Acute leukocyte and temperature response in hypertensive intracerebral hemorrhage / S. Suzuki, R. E. Kelley, B. K. Dandapani, Y. Reyes-Iglesias, W. D. Dietrich, R. C. Duncan // Stroke. — 1995. — № 26 (6). — P. 1020—1023.
62. Zaremba J., Losy J. The levels of TNF-alpha in cerebrospinal fluid and serum do not correlate with the counts of the white blood cells in acute phase of ischaemic stroke / J. Zaremba, J. Losy // Folia Morphol. (Warsz.). — 2001. — № 60 (2). — P. 91—97.
63. Rosenberg G. A., Navratil M. Metalloproteinase inhibition blocks edema in intracerebral hemorrhage in the rat / G. A. Rosenberg, M. Navratil // Neurology. — 1997. — № 48 (4). — P. 921—926.
64. Del Bigio M. R. Experimental intracerebral hemorrhage in rats. Magnetic resonance imaging and histopathological correlates / M. R. Del Bigio, H. J. Yan, R. Buist, J. Peeling // Stroke. — 1996. — № 27 (12). — P. 2312—2319.
65. MacKenzie J. M., Clayton J. A. Early cellular events in the penumbra of human spontaneous intracerebral hemorrhage / J. M. MacKenzie, J. A. Clayton // J.Stroke Cerebrovasc.Dis. — 1999. — № 8 (1). — P. 1—8.
66. Mena H. Human cerebral infarct : a proposed histopathologic classification based on 137 cases / H. Mena, D. Cadavid, E. J. Rushing // Acta Neuropathol. — 2004. — № 108 (6). — P. 524—530.
67. Castle A. G. Properties of p-nitrophenyl phosphatase activity from porcine neutrophils / A. G. Castle, A. Ling, R. Chibber // Int. J. Biochem. — 1984. — № 16 (4). — P. 411—416.
68. Chevrier I. Myeloperoxidase genetic polymorphisms modulate human neutrophil enzyme activity : genetic determinants for atherosclerosis? / [I. Chevrier, D. A. Tregouet, S. Massonnet-Castel, P. Beaune, M. A. Loriot] // Atherosclerosis. — 2006. — № 188 (1). — P. 150—154.
69. Kettle A. J., Winterbourn C. C. A kinetic analysis of the catalase activity of myeloperoxidase / A. J. Kettle, C. C. Winterbourn // Biochemistry. — 2001. — № 40 (34). — P. 10204—10212.
70. Sopata I. Neutrophil enzyme activities in carrageenan-induced inflammation in rats / I. Sopata, E. Wojtecka-Lukasik, J. Wize, S. Maslinski // Agents Actions. — 1989. — № 28 (1–2). — P. 89—92.
71. Turner R. Neutrophil enzyme activities in rheumatoid inflammation / R. Turner, M. Martin, J. Schroff, W. Treadway, L. DeChatelet // Inflammation. — 1979. — № 3 (3). — P. 319—327.
72. Takizawa T. Inflammatory cytokine cascade released by leukocytes in cerebrospinal fluid after subarachnoid hemorrhage / [T. Takizawa, T. Tada, K. Kitazawa, Y. Tanaka, K. Hongo, M. Kameko, K. I. Uemura] // Neurol. Res. — 2001. — № 23 (7). — P. 724—730.
73. Chitnis D. Inhibition of apoptosis in polymorphonuclear neutrophils from burn patients / [D. Chitnis, C. Dickerson, A. M. Munster, R. A. Winchurch] // J. Leukoc. Biol. — 1996. — № 59 (6). — P. 835—839.
74. Barone F. C., Feuerstein G. Z. Inflammatory mediators and stroke : new opportunities for novel therapeutics / F. C. Barone, G. Z. Feuerstein // J. Cereb. Blood Flow Metab. — 1999. — № 19 (8). — P. 819—834.
75. Lampl Y. Prognostic significance of blood brain barrier permeability in acute hemorrhagic stroke / [Y. Lampl, O. Shmuilovich, J. Lockman, M. Sadeh, M. Lorberboym] // Cerebrovasc. Dis. — 2005. — № 20 (6). — P. 433—437.
76. Atzeni F. Induction of CD69 activation molecule on human neutrophils by GM-CSF, IFN-gamma, and IFN-alpha / [F. Atzeni, M. Schena, A. M. Ongari, M. Carrabba, P. Bonara, F. Minonzio, F. Capsoni] // Cell Immunol. — 2002. — 220 (1). — P. 20—29.
77. Hanisch U. K. Microglia as a source and target of cytokines / U. K. Hanisch // Glia. — 2002. — № 40 (2). — P. 140—155.
78. Skinner R. Psychoneuroimmunology of stroke / R. Skinner, R. Georgiou, P. Thornton, N. Rothwell // Neurol. Clin. — 2006. — № 24 (3). — P. 561—583.
79. Kowianski P. Evolution of microglial and astroglial response during experimental intracerebral haemorrhage in the rat / P. Kowianski, Z. Karwacki, J. Dziewiatkowski, [et al.] // Folia Neuropathologica. — 2003. — № 41 (3). — P. 123—130.
80. Power C. Intracerebral hemorrhage induces macrophage activation and matrix metalloproteinages / C. Power, S. Henry, M. R. Del Bigio, P. H. Larsen [et al.] // Annals of Neurology 2003. — № 53 (6). — P. 731—742.
81. Bzowska M. Increased IL-10 production during spontaneous apoptosis of monocytes / M. Bzowska, K. Guzik, K. Barczyk, M. Ernst, H. D. Flad, J. Pryjma // Eur. J. Immunol. — 2002. — № 32 (7). — P. 2011—2020.
82. Wang J., Tsirka S. E. Contribution of extracellular proteolysis and microglia to intracerebral hemorrhage / J. Wang, S. E. Tsirka // Neurocritical Care. — 2005. — № 3 (1). — P. 77—85.