CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1324393

Available online at: http://www.iajps.com Research Article

# INNOVATIVE METHODS OF STUDYING BLOOD WITH ATOMIC FORCE MICROSCOPY IN GERIATRICS

**Tatyana V. Pavlova[1], Victoria V. Bashuk[1], Natalia B. Pilkevich[1], Ivan A. Pavlov[2], Yury A. Lykov[1]**

[1]Belgorod State University, 308015, Belgorod, Pobeda Street, 85, Russia

[2]Belgorod Cancer Clinic, urological department, Kuibysheva str., 1, 308010, Belgorod, Russian Federation, E-mail: pavlova@bsu.edu.ru

**Abstract:**

***Objective:*** *To study the morphological forms of erythrocytes, their pathomorphological changes in diseases of the cardiovascular system, as well as for oncological pathology in the organs of the urinary system with atomic force microscopy (AFM).*

***Methods:*** *The morphological properties of erythrocytes were studied by means of hemoscanning and erythrocytometry. AFM was produced by us in the probe laboratory Ntegra-Aura (Russia). The studies were performed in contact modes of intermittent or constant profiles using commercial Si or SiN cantilevers in conditions of low atmospheric vacuum. Processing and implementation of AFM images were formulated using the software "NOVA" and "ImageAnalysis".*

***Results:*** *It was shown that as the severity and intensity of chronic heart failure (CHF) increased, the altered forms of erythrocytes increased. We revealed an increase in the number of irregular forms of erythrocytes from the total number, the number of cells with the effect of "deflated ball", shadow cells, and the number of red blood cells involved in the processes of sludging. Patients with prostate cancer (PC) and kidney cancer (KC) had a decrease of discocytes in comparison with the control group and the group with benign prostatic hyperplasia (BPH) and an increase of transitional, prehemolitic and degenerative forms. There was no significant difference in the structure of erythrocytes in different age groups.*

***Conclusions:*** *As a result of the study it was established that in CHF and oncological processes a change in the cytoarchitectonics of erythrocytes is observed. Modified cells are partially functionally inferior and cannot fully fulfill their most important oxygen transport function.*

**Key words:** *atomic force microscopy, erythrocytes, diseases of the cardiovascular system, oncology*

*** Corresponding author:**

**Tatyana V. Pavlova,**

*Belgorod State University,*

*308015, Belgorod, Pobeda Street, 85, Russia*

*E-mail: pavlova@bsu.edu.ru*

QR code

*Please cite this article in press Tatyana V. Pavlova et al.,* ***Innovative Methods of Studying Blood with Atomic Force Microscopy in Geriatrics.,*** *Indo Am. J. P. Sci, 2018; 05(07).*

## INTRODUCTION:

Aging is a collection of different physiological changes that can be useful, neutral or unfavorable for organism [1]. Negative physiological changes are combined with changes in adaptive processes, which lead to the accumulation of damage, and there is also a reduction in the reserve capacity of functioning [2]. At the same time, the aging organism will observe qualitative and quantitative changes in the biological indices of vital activity, which may increase, decrease or remain at a stable level [2, 3]. Knowledge of age-related changes and reference values in geriatrics when examining elderly patients will allow timely action to improve the quality of life and prevent decompensation of existing diseases. With aging, somatic pathology arises and progresses [3].

The analysis of the structure of polymorbidity in elderly patients has shown that most often urological pathologies are accompanied by diseases of the cardiovascular system, as well as lesions of the endocrine, nervous systems, somewhat less often in the gastrointestinal tract and reproductive system [4, 5]. Concomitant diseases can lead to the emergence and progression of previously acquired functional urological disorders, while the reciprocal influence of involutive processes on the course and prognosis of the existing pathology is not excluded [5].

To date, the most relevant problem is the tumor lesion of the urinary system. The search for markers of processes of physiological and pathological aging is due to a stable world trend towards an increase in the proportion of people older than working age and features of the demographic situation in the Russian Federation [6-8].

Due to the wide prevalence and social importance, the range of research and diagnostics in geriatrics, in particular, in oncology, including morphology, is increasing [9-11].

Atomic force microscopy (AFM) can be effectively used as a screening method. With its help, it is possible to observe all possible structural features in pathological conditions [12-13]. Atomic force microscopy (AFM) provides 3D images of surface ultrastructure with molecular resolution in real time [13-16].

Thus, the purpose of our survey was to study the morphological forms of erythrocytes in the pathology of the cardiovascular system and oncological processes of the urinary system in geriatric aspects with the help of Atomic force microscopy (AFM).

## MATERIALS AND METHODS:

Within the framework of the work done, the study included:

Control group:

1) Almost healthy middle-aged people (n = 33, age from 40 to 59, average age 48.2±2.5 years, 15 men, 18 women);

2) Almost healthy people of elderly and senile age (n = 31, age from 60 to 79 years, average age 68.7 ± 2.2 years, 15 men,16 women).

Patients with CHF FC:

• I (n = 63): 1) middle-aged patients with CHF FC I: (n = 31, age 40 to 59 years, mean age 4.2 ± 3.0 years, 20 men, 11 women). 2) elderly and senile patients with CHF FC I (n = 32, age 60 to 79 years, mean age 69.4 ± 2.4 years, 20 men, 12 women).

• II-III (n = 65): 1) middle-aged patients with chronic heart failure FC II-III (n = 32, age 40 to 59 years, mean age 51.1 ± 2.8 years, 20 men, 12 women); 2) elderly and senile patients with chronic heart failure FC II-III (n = 33, age 60 to 69, mean age 71.3 ± 2.4 years, 21 men, 12 women).

• IV (n = 66): 1) middle-aged patients with chronic heart failure FC IV (n = 31) (age from 40 to 59 years, mean age 52.0 ± 3.3 years, 23 men, 8 women). 2) elderly and senile patients with chronic heart failure FC IV (n = 35, age 60 to 79 years, mean age 71.2 ± 2.4 years, 26 men, 9 women).

Patients with kidney and prostate pathology:

• First group included 110 men with prostate cancer (PC) and benign prostatic hyperplasia (BPH).

• Second group included 88 men and women suffering from kidney pathology: kidney cancer (KC) and kidney cysts.

Object of the study - blood samples from vein.

### 2.1. Study of the morphological characteristics of erythrocytes

The material for the study was native red blood cells. Venous blood was taken in the morning on an empty stomach in vacuum test tubes. During the first 30 minutes, a sequence of washing the erythrocyte mass was carried out, and blood smears were formed. The morphological properties of erythrocytes in the obtained samples were studied by means of hemoscanning and erythrocytometry.

### 2.2. Study of pathomorphological changes in cells and tissues

Cells and tissues were analyzed using atomic force microscopy (AFM). Samples were viewed and photographed after a brief fixation in formalin solution. It makes possible to use atomic force microscopy (AFM) as an express method. The studies were performed in contact modes of intermittent or constant profiles using commercial Si or SiN

cantilevers (NSG01, NT-MDT, Russian Federation) under conditions of low atmospheric vacuum. AFM images were processed and implemented using the NOVA software (NT-MDT, Russian Federation) and ImageAnalysis (NT-MDT, Russian Federation).

## RESULTS AND DISCUSSION:

### 3.1. Pathophysiological and morphometric features of erythrocytes

The results of hemoscanning, carried out with the help of atomic force microscopy for the evaluation of the structural and functional characteristics of blood cells, showed that in patients as the age increased, the following pattern is observed. The averaged cell size was 6.12 ± 0.30 μm. They were mostly in the form of a regular oval. In erythrocytes with outgrowths on the surface their value was 672.45 ± 50.30 μm. The content of unchanged discocytes decreased from 23.0 ± 0.2% in almost healthy middle-aged people and to 15.0 ± 0.4% in elderly recipients. A similar picture was observed for transitional forms (ellipses, discocytes: with a crest, flat discocytes, with an outgrowth, with multiple outgrowths, erythrocytes in the form of a "mulberry berry"), which was especially typical for erythrocytes with multiple outgrowths (from 72.5 ± 0.3% to 51.3 ± 1.3%). The content of dome-shaped, spherical cells also increased in the form of a "deflated ball", as well as degenerative forms. Most of the red blood cells had a rounded shape (Fig. 1). However, we also observed cells of elongated shape, which can indicate both a violation of membrane elasticity and difficulty of passage through the microcirculatory bed due to a change in its structure, which was more typical for erythrocytes in elderly patients. Erythrocytes with the phenomena of sludge (7.0 ± 0.6% and 8.2 ± 0.8%) and shadow cells (3.9 ± 0.2% and 4.6 ± 0.7%) were found in the fields of vision.

Analysis of the percentage ratio of erythrocytes showed that in all studied age groups the prevalent content was in erythrocytes, their diameter was 6.8-8.2 μm (normocytes). In the group of almost healthy people, the normocytes were 75.4 ± 3.11% and 72.5 ± 3.10%. The content of microcytes in almost healthy people was 22.6 ± 3.15% and 23.5 ± 3.15%. In control group, macrocytes content was 2.0 ± 028% and 3.0 ± 0.21%. The mean erythrocyte diameter was 7.10 ± 0.05 μm and 7.40 ± 0.06 μm.

The following changes were observed in scanning erythrocytes in middle-aged patients with chronic heart failure FC I: 86.1 ± 0.4% of erythrocytes were represented by the following variants of discocytes: without outgrowths (31.0 ± 0.2%), with one outgrowth (6.6 ± 04%), with crest (7.0 ± 0.2%). The content of «mulberry» erythrocytes increased (3.5 ± 0.3%); the number of erythrocytes with multiple outgrowths decreased (37.2 ± 0.6%). The remaining 13.9 ± 0.2% of erythrocytes were dome-shaped, spherocytes: with a smooth surface, with spinules on the surface, erythrocytes in the form of a "deflated ball." A similar dynamic was obtained with blood scanning in elderly people suffering from CHF I FC. When scanning erythrocytes in patients with CHF I FC of the elderly, the following changes were observed: 80.8 ± 0.7% of erythrocytes were represented by discocytes without outgrowths (35.6 ± 0.3%), with one outgrowth (7.2 ± 0.4%), with a crest (5.0 ± 0.3%). The content of erythrocytes in the form of mulberry (5.0 ± 0.4%) increased. The number of cells with multiple outgrowths decreased (35.5 ± 0.7%). Remaining 19.2 ± 0.2% were domed erythrocytes, spherocytes with a smooth surface and with spinules. The content of erythrocytes in the form of a "flat ball" (2.8 ± 0.2% of erythrocytes from the total number of scanned cells) increased, the number of cells of irregular shape increased (up to 6.7 ± 0.3%, $p < 0.05$ compared to almost healthy elderly people). The number of cells-shadows did not differ significantly ($p > 0.05$) from their content in the blood of healthy people and was 3.7 ± 0.2% of the total number of scanned erythrocytes. On the surface of erythrocytes, the number of outgrowths, as well as their dimensions (322.30 ± 61.30), significantly decreased. On the surface of erythrocytes, the number of pores was reduced, and the remaining ones were of smaller size and shape.

More pronounced changes were observed when scanning erythrocytes in patients with CHF II-III FC. Thus, in middle-aged people suffering from CHF II-III FC, the number of reversibly deformed forms increased to 76.8 ± 0.6%. They were distributed as follows: discocytes without outgrowths - 38.7 ± 0.5%, with one outgrowth - 6.3 ± 0.6%, with a ridge - 6.4 ± 0.4%. The content of erythrocytes in the form of mulberry (6.6 ± 0.4%) increased. The number of erythrocytes with multiple outgrowths (20.3 ± 0.8%) reduced (Fig. 2).

Altered discocytes (34%) in the main group were distributed among themselves as follows: about 19% of the red blood cells were spherocytes with spines on the surface, 11% in the shape of "deflated ball" and degenerate cells. The remaining 4% were represented by domed erythrocytes and spherocytes with a smooth surface. Also microcytomas were observed, but there were more macrocytes. The shape of the cells was significantly changed. On average, the cell sizes were 7.2 ± 0.32 μm. The size of outgrowths on the surface of cells decreased.

In elderly people suffering from CHF II-III FC, the following changes were observed. The number of erythrocytes with the effect of a " deflated ball " increased to 5.6 ± 0.2% of the total number of scanned erythrocytes, sludging increased 15.4 ± 0.3%, the number of irregular forms of erythrocytes also increased - 12.4 ± 0.2%, the number of shadow cells increased to 5.3 ± 0.1% (for all indicators p <0.05 compared with elderly patients suffering from CHF I FC). At the same time, the number of irregular forms of erythrocytes and the degree of sludging were significantly higher (p <0.05) than in middle-aged people with CHF II-III FC. The study of blood samples obtained from patients with CHF IV FC showed that in middle-aged people suffering from CHF IV FC, the following changes were observed. When evaluating the structural and functional characteristics of erythrocytes, discocytes without outgrowths (35.4 ± 1.2%), discocytes with one outgrowth (4.5 ± 0.2%), less often discocytes with a crest (6.2 ± 0.3%), discocytes with multiple outgrowths (20.5 ± 1.6% %) and individual erythrocytes in the form of mulberry (4.9 ± 0.1%). The first five classes of erythrocytes accounted for 69%. The remaining 31% were in the form of spherical cells with a smooth surface, spherocytes with spines on the surface, erythrocytes in the form of a "deflated ball." They belong to the group of irreversibly deformed or prehemolitic erythrocytes. Average cell sizes were smaller than in other groups. They were 5.74 ± 0.42 μm. They were mostly in the form of a regular oval. In erythrocytes with outgrowths on the surface their value was 494.43 ± 39.50 μm. It was also less than in other groups. At the same time, we should note the sluggish phenomenon, as well as an increase in the content of shadow cells.

In elderly people with CHF IV FC, the following changes were observed. When evaluating the structural and functional characteristics of erythrocytes, it was shown that there were predominantly discocytes without outgrowths (31.8 ± 1.1%), discocytes with one outgrowth (4.3 ± 0.3%), less often discocytes with a crest (6.5 ± 0.3%), discocytes with multiple outgrowths (19.6 ± 0.9%) and individual erythrocytes in the form of mulberry (5.0 ± 0.2%), which were often fragmented. The first five classes of erythrocytes were 65% (for all indices p <0.05 compared with elderly patients with hypertension arterial hypertension (AH) and p> 0.05 compared with middle-aged patients with AH and ischemic heart disease (IHD)). Remaining 35% were in the form of spherical cells with a smooth surface, spherocytes with spinules on the surface, erythrocytes in the form of a "deflated ball." They belong to the group of irreversibly deformed or prehemolitic cells. Cell sizes were on average smaller than in other groups. In this case, they were 5.17 ± 0.33 μm. There were mostly in the form of a regular oval. In erythrocytes with outgrowths on the surface their value was 486.55 ± 43.20 μm, and it was also less than in other groups. The content of shadow cells increased. At the same time, the number of irregular forms of erythrocytes and the degree of sludging was significantly higher (p <0.05) than in middle-aged people with chronic heart failure IV FC.

In patients with CHF I-IV FC in erythrocytes, the propensity to stasis, sludge phenomenon and thrombosis increased with increasing severity of the disease. The number of cytoplasmic bridges between the cells, as well as the filaments of fibrin, increased. In addition, these indicators of circulatory disturbances progressed as the age of the patients increased. In comparison with almost healthy people and with CHF I FC in patients of middle age in the group of elderly people, erythrocytes often took the form of an elongated oval.

It was shown that the depth of the discocyte hollow, calculated by studying the profile of the cell, averaged 0.25 ± 0.06 μm and 0.42 ± 0.06 μm (p <0.05). When studying the ratio of the erythrocyte diameter to the diameter of the cavity, this value was found in the limits of 19.0 ± 3.0 and 18.0 ± 2.0 units. The pore structure was changed on the erythrocyte membranes in groups with elderly patients. When studying the surface of erythrocytes in patients with CHF I-IV FC, it was shown that the depth of the discocytes hollow varied in rather wide limits and did not directly depend on the group (0.32 ± 0.21 μm) (p <0.05), as well as the ratio of erythrocyte diameter to the diameter of the cavity. The structure of the pores on membranes of erythrocytes has been significantly altered, especially in groups with elderly patients.

When studding the morphological state of erythrocytes in the first group of patients with diagnosed prostate cancer (PC) and benign prostatic hyperplasia (BPH) compared with the control group showed that in patients with benign prostatic hyperplasia (BPH) we noticed a significant (p <0.05) decrease 5.77% in of the number of discocytes and 16.67% of irreversibly altered erythrocytes. At the same time, reversibly altered erythrocytes increased 1,5 times, and erythrocytes with degenerative forms 2,5 times.

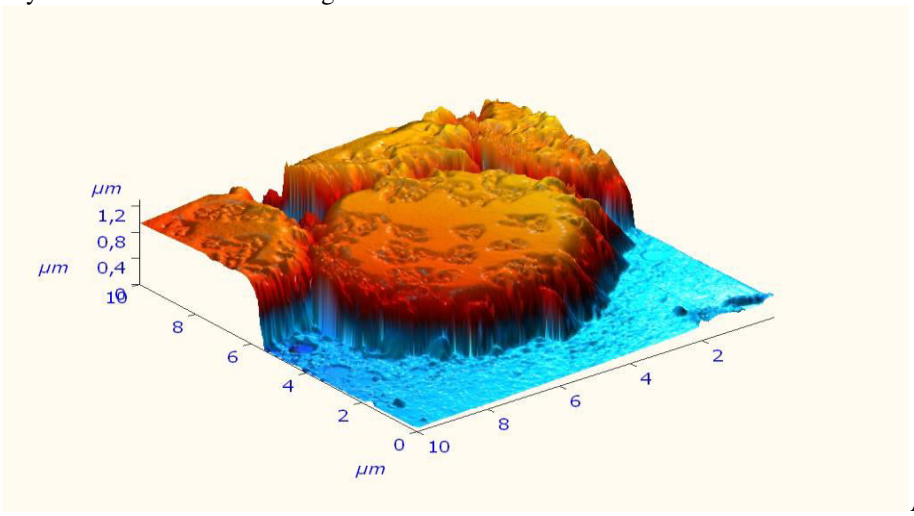
A detailed analysis of erythrocyte architectonics in the prostate cancer (PC) patient group reflected the morphological heterogeneity of the erythrocyte population. A significant decrease in discocytes was

observed at 17.31% (71.67 ± 2.53%) in comparison with the control group and at 12.25% compared with patients with benign prostatic hyperplasia (BPH). An increase in the transient forms of erythrocytes – 2,3 times, prehemolitic – 1,33 times, and degenerative – 3,5 times in comparison with the control group. In its turn, in 1,5, 1,6 and 1,4 times, respectively, compared with patients with benign prostatic hyperplasia (BPH) (Fig. 3).

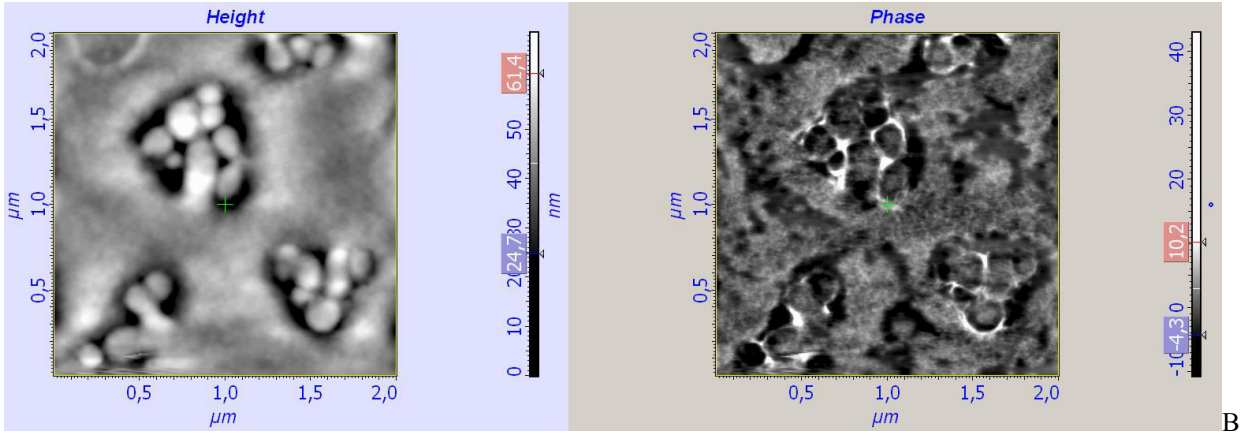
The study of the morphological state of erythrocytes in the group of patients with kidney cancer (KC) compared with the group of patients with kidney cysts showed that in patients with kidney cancer (KC) there was a significant ($p < 0.05$) decrease in 5.53% of the number of discocytes, 4.81% of the number of irreversibly altered and 14.53% of degenerative forms of red blood cells. At the same time, we observed a significant increase (34,5%) in reversibly altered erythrocytes. There was no significant difference in the structure of erythrocytes in different age groups.

Thus, it was shown that as the severity and intensity of chronic heart failure increased, the altered forms of erythrocytes increased. We reavealed an increase in the number of irregular forms of erythrocytes compared to the total number of RBC, the number of cells with the effect of a "deflated ball", shadow cells, and the number of red blood cells involved in the processes of sludging.

In patients with prostate cancer (PC) and kidney cancer (KC), a decrease in discocytes was observed in comparison with the control group and group with benign prostatic hyperplasia (BPH), an increase in transient, prehemolitic and degenerative forms. There was no significant difference in the structure of erythrocytes in different age groups.

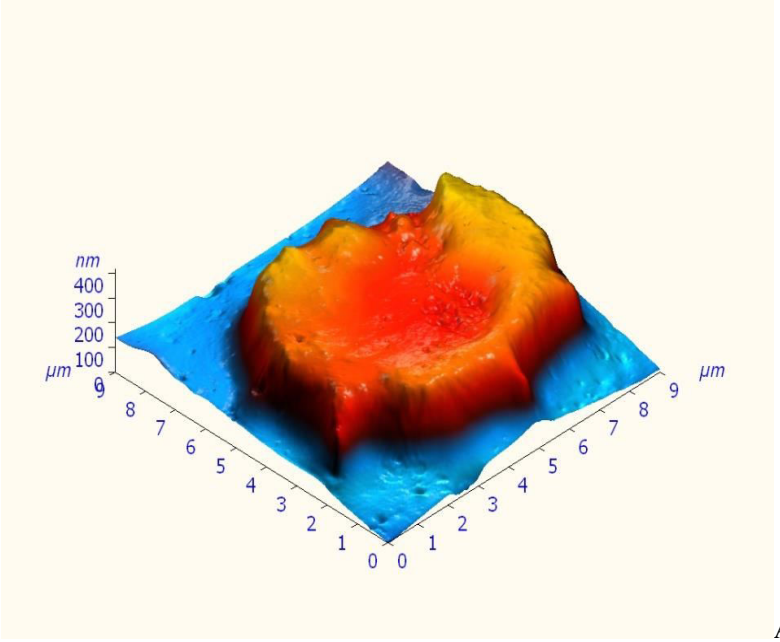



A

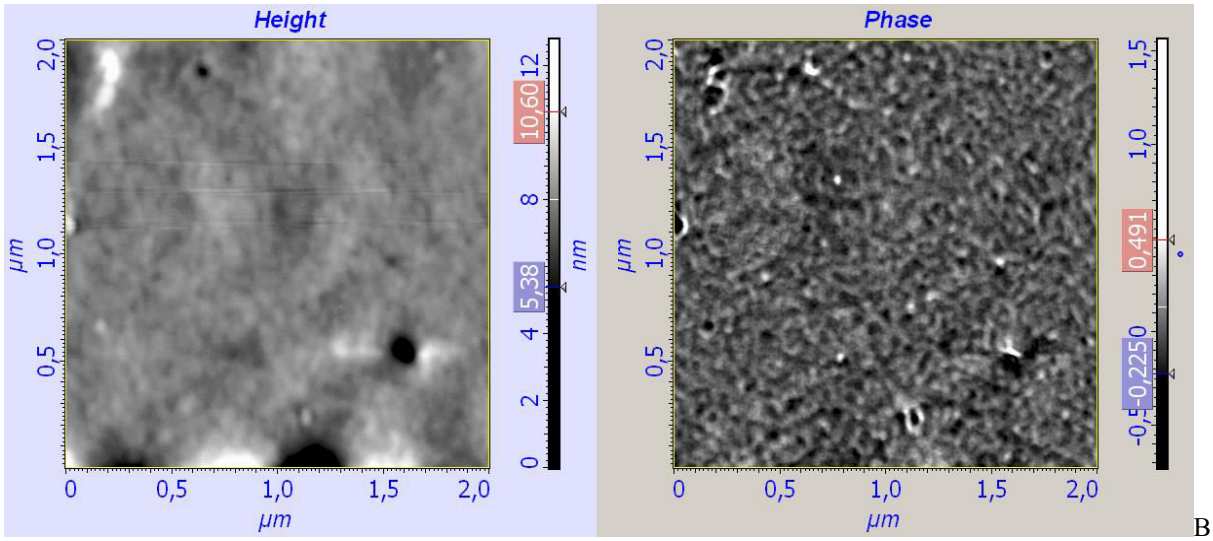



B

**Figure 1.** Erythrocytes of blood in almost healthy middle-aged people. Discolets with multiple outgrowths (A). Pores on the surface of erythrocytes are clearly contoured (B). Atomic force scanning microscopy. A. Three-dimensional histogram. B. Two-dimensional histogram. Surface of erythrocytes.

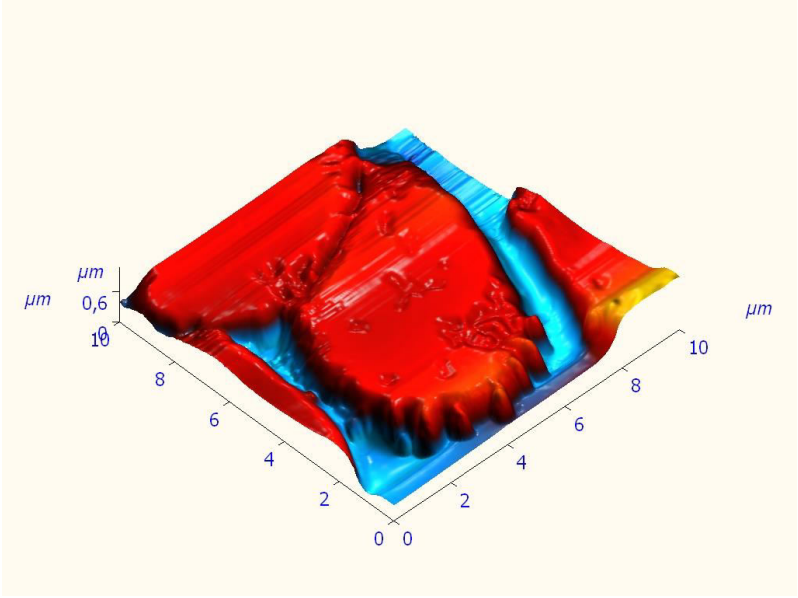



A

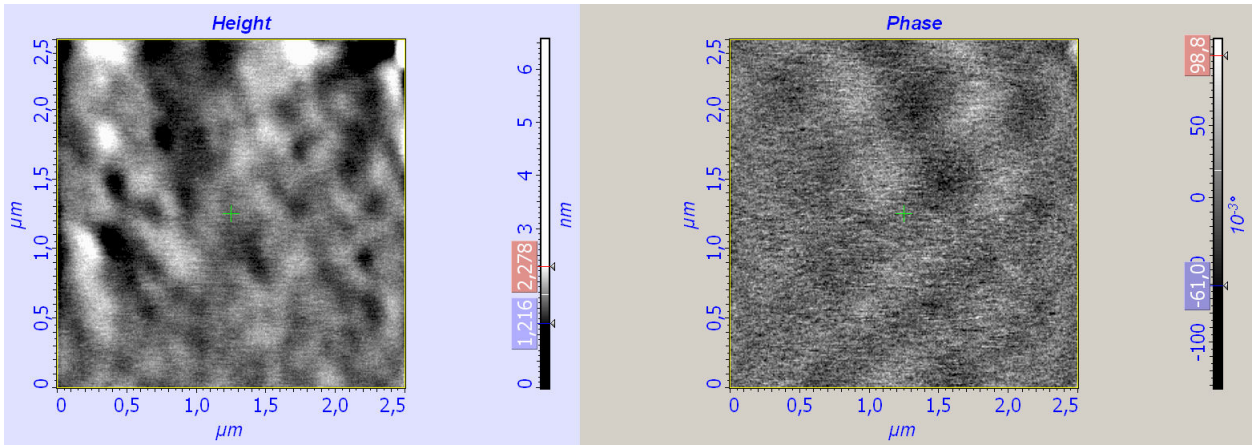



B

**Figure 2.** Erythrocytes in patients with chronic heart failure of the second and third functional class. Change in the shape of cells (A). Surface disturbance and change in the relief of pores (B). Atomic force scanning microscopy. A. Three-dimensional histogram. B. Two-dimensional histogram.




A




B

**Figure 3.** Erythrocytes of blood with diagnosed prostate cancer

The shape and dimensions of the cells are changed. Disturbance of the diameter of the erythrocyte cavity (turquoise color). Change in the structure of the plasma membrane. Individual cells are hemolyzed (pink). Violation of the structure of pores and cytoplasmic processes on the surface of cells.
Atomic power laboratory. A. Three-dimensional bar graph. B. Two-dimensional histogram.

## CONCLUSION:

Thus, using atomic force microscopy (AFM), it was shown that with increasing of severity and intensity of chronic heart failure, the altered forms of erythrocytes increased. An increase in the number of irregular forms of erythrocytes from the total number, the number of cells with an effect of a "deflated ball", shadow cells, and the number of red blood cells involved in the processes of sludging have been revealed.

In patients with prostate cancer (PC) and kidney cancer (KC) we observed a decrease in discocytes in comparison with the control group and group with benign prostatic hyperplasia (BPH) and an increase in transient, prehemolitic and degenerative forms. There was no significant difference in the structure of erythrocytes in different age groups.

The possibility of studding using atomic force microscopy (AFM) in diagnostic, screening studies and as an express method makes it attractive for pathologists, cytologists and oncologists.

## REFERENCES:

1. Shabalin, V.N., 2009. Organization of work of the geriatric service in the conditions of the progressive demographic aging of Russian

Federation population. *The successes of gerontology*, 1 (22):186-195.
2. Shlyafer, S.I., 2013. Modern demographic situation of the aging of the population of Russia. *Glavvrach*, 1:39-46.
3. Bucher, S., 2016. Current trends in aging of the population of Russia. *Bulletin of the Russian Academy of Sciences*, 3(86):215-223.
4. Vorobiev, A.V., 2006. Review of the most important events in oncourology. *Practical oncology*, 6(1):55-64.
5. Welscher, L.Z., Polyakov, B.I., Peterson, S.B., 2009. Clinical Oncology: Selected lectures. M.: GEOTAR-Media, 404-423.
6. Chaplygina, M.A., Kharchenko, Y.A., Pavlova, T.V., 2014. Innovative methods of research in oncology. *International Journal of Experimental Education*, 5(1):36-40.
7. Pavlova, T.V., Chaplygina, M.A., Pavlov, I.A., 2014. New in the diagnosis of prostate cancer in elderly patients. *Vrach*, 6:36-38.
8. Pavlova, T.V., Kulikovsky, V.F., Pavlova, L.A., 2016. Clinical and experimental morphology. M.: «*Medical Information Agency*», :256.
9. Pavlova, T.V., Bessmertnykh, D.V., Pavlov, I.A., 2010. Modern aspects of treatment and assessment of the quality of life of elderly patients with prostate cancer. *Scientific bulletins of the Belgorod State University*, 22 (93):67-71.
10. Pavlova, T.V., Bessmertny, D.V., Pavlov, I.A., 2010. Modern aspects of treatment and evaluation of the quality of life in patients with prostate cancer in the elderly. *Scientific bulletins of the Belgorod State University*, 12 (33):15-16.
11. Ando, T., 2012. High-speed atomic force microscopy coming of age. *Nanotechnology,* 11:201-206.
12. Baro, A.M., Reifenberger, R.G., 2012. Atomic force microscopy in liquid. *Biological Applications,* :402.
13. Pavlova, T., Pavlov, I. A., Pilkevich, N. B., Pavlova, I. A., Goncharov, I. Y., Bashuk, I. P., 2017. Methods of atomic-force microscopy within the scope of research of oncological processes. Virchows Archiv. The European Journal of Pathology. 29th European Congress of Pathology. Pathology for Patient Care 2-6 September 2017. RAI Amsterdam, The Netherlands. *Virchows Arch,* 471 (Suppl 1): S1–S352.
14. Pavlova, T., Bessmertny, D., Pavlov, I., Lykov, Y., 2015. Scanning microscopy as diagnostic test of cancer of organs of urinary system Virchows Archiv, 467 (Suppl 1): S1-233.
15. Chaplygina, M.A., Pavlova, T.V., Proshchayev, K.I., 2013. New approaches in the study of blood cancer patients. *Gerontology: electron. sci. magazine*, 1(3).
16. Prashchayeu, K., Pozdnyakova, N., Bashuk, V., Selivanov, A., 2013. Erythrocytes as target cells of diabetes types 1 and 2. Virchows Archiv. The European Journal of Pathology. 25th European Congress of Pathology. European Society of Pathology: 157-158.