FEATURES OF BLOOD IN ONCOUROLOGICAL PATHOLOGY

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Abstract:
*Objective:*To study the morphological forms and the pathomorphological changes of erythrocytes in oncological processes 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:*The number of microcytes in patients with kidney diseases is lower than in groups of almost healthy people of both ages and with the pathology of the prostate gland. There was a reliable increase in the content of irreversibly altered forms of erythrocytes in the group of almost healthy elderly people. In BPH, compared with almost healthy people, there was a decrease in the number of discs and an increase in reversibly altered forms (due to a pronounced increase in the content of discocytes with multiple outgrowths). For a group of patients with diagnosed prostate cancer, a decrease in the level of discocytes and an increase in the content of transitional forms were characteristic, due to an increase in the percentage of discs with multiple outgrowths.
*Conclusions:*As a result of the study it was established that in oncological processes a change in the cytoarchitectonics of erythrocytes is observed. Modified cells are partially functionally inferior.
Key words: *erythrocytes, scanning electron microscopy, atomic force microscopy, prostate cancer, kidney cancer.*

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INTRODUCTION:

To date, blood is the most diagnosed system and represents an organ on which many pathogenetic and sanogenic mechanisms in the human body are closed [9]. Both the morphological and functional components of blood, as an organ, are involved in processes associated with oncopathology[1,2,3]. Increased interest of researchers in erythrocytes is due to the participation of the latter in the processes associated with maintaining homeostasis at the level of the whole organism. Now a number of studies indicate the involvement of red blood cells in pathological processes, not only in hematological diseases. The obtained results reflect the patterns of changes in the structure and function of erythrocytes in various diseases. The question of studying the width of distribution of erythrocytes by volume gains more and more urgency. The results of the studies show the possibility of using this criterion as a prognostic marker [4,5,6,7,8]. The relationship between the distribution width of red blood cells and the mortality of elderly patients with both age-associated pathology and without it was also determined. Thus, the parameters of erythrocytes can be used as a diagnostic and prognostic criterion. The study of changes in erythrocytes in oncopathology can underlie the pathogenetic foundation of the principles of restoring the functional properties of cells and metabolism in general.

MATERIAL AND METHODS:

The study involved 58 patients (39.7% of women, 61.3% of men) who formed 6 groups. The first two groups are almost healthy patients of average (44.50 ± 2.01) and elderly (64.70 ± 1.84) age respectively, who do not have severe somatic pathology, exacerbations of chronic forms of diseases and with a favorable prognosis. The third group consisted of patients with benign prostatic hyperplasia (BPH) (64.50 ± 2.91), the fourth group with primary diagnosed verified prostate cancer (67.80 ± 2.56). The fifth group consisted of patients with a kidney cyst (62.80 ± 2.06), in the sixth patients with verified kidney cancer (63.30 ± 2.73). In this case, prostate cancer in most cases (80%) is represented by small acinar adenocarcinoma and malignant neoplasms of the kidney is represented by clear cell cancer (90%).

Venous blood served as a material for the study. The material was collected in the morning on an empty stomach in vacuum tubes with the addition of heparin. Within 30 minutes, a procedure was carried out for sequential washing of the erythrocyte mass, and the formation of blood smears. Description of the morphometry and photographing of cells in the obtained samples was performed in a scanning microscope «FE1 Quanta 200 3D». In addition, the cells were studied using the atomic force microscope "Ntegra-Aura". Studies were carried out in the modes of permanent or intermittent contacts. The processing and construction of AFM images was carried out with the help of NOVA software (NT-MDT, Russia) and Image Analysis (NT-MDT, Russia). The obtained sizes of erythrocytes were divided into 3 groups: microcytes (up to 6.4 μm), normocytes (6.5-8.9 μm), macrocytes (9.0-10.9 μm), megalocytes (more than 10.9 μm). When describing the morphological characteristics, the erythrocyte population was divided into the main groups: 1) discocytes; 2) transitional forms (capable of reverse transformation): ellipses, discocytes with a crest, flat discs, discocytes with an outgrowth, discocytes with multiple outgrowths, erythrocytes in the form of a "mulberry"; 3) pre-hemolytic forms (with changes that are irreversible): domed, spherical, in the form of a "deflated ball"; 4) degenerative forms.

Statistical analysis of the study results was carried out on a personal computer (IBM PC - Intel Pentium-IV). During the statistical processing of data, intensive and extensive indices of average values were calculated. The reliability of differences in the mean and relative quantitative values was determined by the Student's t-test. Differences were considered reliable, provided that the level of reliability was p <0.05. Calculations were carried out using Microsoft Excel software, using the Statistica 6.0 package.

RESULTS AND DISCUSSION

The data obtained with the help of scanning electron microscopy in control groups and groups of patients with urologic and oncourologic pathology is presented in Table 1., Fig. 1.

Thus, in the group of almost healthy middle-aged people, a large percentage of the erythrocyte population was represented by normocytes - 83.3 ± 1.43%, the share of microcytes accounted for 16.3 ± 1.52%, and macrocytes were 0.4 ± 0.14%. The mean size of red blood cells was 7.10 ± 0.04 μm. The diameter of microcytes was 6.1 ± 0.04 μm, the normocytes were 7.2 ± 0.04 μm, the average size of macrocytes was 9.2 ± 0.07 μm. When determining the ratio of the morphological forms of erythrocytes, the prevalence of discocytes was 88.67 ± 2.44%. The content of transitional forms capable of reverse transformation (ellipses, discocytes with a crest, flat discs, discocytes with an outgrowth, discocytes with multiple outgrowths, erythrocytes in the form of a "mulberry") was 11.0 ± 2.39%, prehemolithic (irreversible) forms (domed, spherical, in the form of a "deflected ball") - 0.17 ± 0.17%, and the same number of degenerative ones.

The study of erythrocytes in a group of almost healthy elderly people showed that the predominant percentage of red blood cells also accounted for normocytes (75.6 ± 3.14%), while their content is lower than in the middle age group, and the number of microcytes is reliably increased (23.3 ± 3.28%). Macrocytes were observed in 1,1 ± 0,28% of the total number of scanned cells.

Cytometric analysis showed that the average size of erythrocytes was 7.00 ± 0.05 μm. The diameter of microcytes in the elderly age was 5.9 ± 0.06 μm, which is reliably lower relative to the control group of middle age ($p < 0.05$). The diameter of normal cells was 7.3 ± 0.04 μm, and the average size of macrocytes was 9.2 ± 0.07 μm. The results of the study of the morphological characteristics of erythrocytes in elderly people showed the following ratio of the main forms: discocytes - $86.67 \pm 0.80\%$; transition forms - $10.00 \pm 0.52\%$; pre-hemolytic - $3.00 \pm 0.37\%$; degenerative forms of erythrocytes - $0.33 \pm 0.21\%$. There was a reliable ($p < 0.05$) increase in the percentage of irreversibly altered erythrocytes ($3.00 \pm 0.37\%$) compared with a group of almost healthy middle-aged people. Also, in this group there were single dome-shaped red blood cells and erythrocytes in the form of a "deflated ball".

Morphometric analysis of data in the group of patients with BPH reflected the presence of relative microanizocytosis. The ratio of erythrocytes in size was as follows: microcytes- $35.9 \pm 3.70\%$; normocytes - $63.7 \pm 3.62\%$ and macrocytes- $0.4 \pm 0.14\%$. At the same time, there was a reliable ($p < 0.05$) increase in microcytes and a decrease ($p < 0.05$) in normocytes, relative to groups of patients with kidney diseases. Similarly, there was a reliable ($p < 0.05$) decrease in the content of macrocytes relative to groups of patients suffering from a kidney cyst. In BPH, the mean size of red blood cells was 6.80 ± 0.05 μm. The diameter of microcytes is 6.0 ± 0.04 μm, which is reliably ($p < 0.05$) less than this figure in the group of almost healthy middle-aged people and groups of patients with kidney pathology, and more in comparison with the diameter of microcytes in patients with prostate cancer ($p < 0.05$). The diameter of normal cells was reliably less ($p < 0.05$) than in kidney diseases and was 7.3 ± 0.04 μm. The mean size of the macrocytes was smaller relative to all the study groups (9.0 ± 0.06 μm), but reliably ($p < 0.05$) compared with the groups of patients with prostate cancer and kidney disease.

The study of results of the morphological state of erythrocytes revealed a change in the distribution of cellular forms. There was a reliable ($p < 0.05$) decrease in the number of discs ($81.67 \pm 1.20\%$), compared with control groups of both ages ($p < 0.05$), and an increase in patients with malignant neoplasms of the kidney and prostate. There was also an increase in reversibly altered forms - $15.00 \pm 1.15\%$ in comparison with control groups of both ages, with a group of patients with a kidney cyst, and a decrease in patients with oncological pathology ($p < 0.05$). An increase in the content of transient groups of erythrocytes was due to a reliable ($p < 0.05$) increment in the content of discocytes with multiple outgrowths ($13.17 \pm 1.33\%$). Prehemolytic forms were $2.50 \pm 0.56\%$, which is reliably ($p < 0.05$) higher in comparison with the group of almost healthy middle-aged people. This increase was mainly due to a

reliable increase in the erythrocyte content of the spherical form - $1.50 \pm 0.34\%$ ($p < 0.05$). The number of degenerative forms was $0.83 \pm 0.31\%$.

The results of hemoscanning showed that in the group of patients with diagnosed prostate cancer the erythrocyte mass is characterized by relative anisocytosis. There was a decrease in the number of normocytes $61.9 \pm 2.96\%$: $p < 0.05$ in comparison with control groups of both ages, and $p > 0.05$ relative to groups with kidney diseases. The existing anisocytosis appeared due to the increase of macro - and mainly microcytes (relative microanizocytosis), while the level of macrocytes was $3.7 \pm 1.35\%$, microcytes - $34.4 \pm 3.71\%$. The average size of red blood cells was 6.80 ± 0.06 μm. A comparative analysis of the size of microcytes showed a decrease in diameter for all study groups (5.8 ± 0.06 μm): $p < 0.05$ in comparison with almost healthy middle-aged people, patients with kidney pathology and patients with prostate cancer. The diameter of normocytes was 7.3 ± 0.05 μm. The average size of macrocyteswas 9.5 ± 0.08 μm, which is reliably ($p < 0.05$) more, in comparison with control groups of both ages. In patients with prostate cancer, in comparison with the control group of the elderly, there were erythrocytes in diameter less than 4.0 μm.

In the group of patients with prostate cancer there is a morphological heterogeneity of the erythrocyte population. There is the most pronounced decrease in discocytes ($71.67 \pm 2.53\%$), reliably relative to groups of almost healthy people of both ages, as well as patients with BPH, kidney cyst ($p < 0.05$). Prehemolytic forms were $4.00 \pm 0.77\%$ (reliable ($p < 0.05$) increase in comparison with the group of almost healthy middle-aged people), and degenerative -$1.17 \pm 0.48\%$. There was a reliable increase in the number of transient forms of erythrocytes ($23.17 \pm 1.89\%$) relative to groups of almost healthy people of both ages, as well as patients with BPH, kidney cyst ($p < 0.05$). The percentage increase in the group of reversibly altered erythrocytes was due to a reliable ($p < 0.05$) increase in the number of discs with multiple outgrowths ($21.00 \pm 1.86\%$) compared to all study groups.

Table 1: **Characteristics of erythrocytes sizes**

Erythrocyte size ratio	The group of almost healthy people		Groups of patients			
	middle-aged *(44.50±2.01)*	elderly *(64.70±1.84)*	Patients with BPH *(64.50±2.91)*	patients with prostate cancer *(67.80±2.56)*	Patients with kidney cyst *(62.80±2.06)*	Patients with kidney cancer *(63.30±2.73)*
Microcytes (%)	16.3±1,52	23.3±3,28	35.9±3,70 ^{xx}	34.4±3.71 ^{xx}	0.4±0.14 $^{*, **, +, ++, xx}$	5.2±0.34 $^{**,+,++,x}$
Normocytes (%)	83.3±1.43	75.6±3.14	63.7±3.62 $^{x, xx}$	61.9±2.96 $^{x, xx}$	91.9±1.08 $^{+,++}$	93.3±0.47 $^{+,++}$
Macrocytes (%)	0.4±0.14	1.1±0.28	0.4±0.14 ^{x}	3.7±1.35	7.8±0.95 $^{*, **, +, xx}$	1.5±0.27 ^{x}
Size of red blood cells						
Erythrocytes diameter (μm)	7.1±0.04	7.0±0.05	6.80±0.05	6.80±0.06	6.80±0.06	7.90±0.05
Microcytes diameter (um)	6.1±0.04	5.9±0.06 *	6.0±0.04 $^{*, ++, x, xx}$	5.8±0.06 $^{*, +, x, xx}$	6.4±0.07 $^{*,**,+, ++}$	6.2±0.05 $^{**,+, ++}$
Normocytes diameter (um)	7.2±0.04	7.3±0.04	7.3±0.04 $^{x, xx}$	7.3±0.05 ^{x}	7.8±0.04 $^{*,**,+, ++, xx}$	7.4±0.03 $^{*,**,+, x}$
Macrocytes diameter (um)	9.2±0.07	9.2±0.07	9.0±0.06 $^{++,x, xx}$	9.5±0.08 $^{*,**,+}$	9.5±0.08 $^{*,**,+}$	9.7±0.19 $^{+}$
Morphological forms						
Discocytes	88.67±2.44	86.67±0.80	81.67±1.20 $^{*,**,++,xx}$	71.67±2.53 $^{*,**,+,x}$	81.50±1.93 $^{*,**, ++}$	77.00±0.68 $^{**,+}$
Reversibly Modified	11.00±2.39	10.00±0.52	15.00±1.15 $^{*,**,++,xx}$	23.17±1.89 $^{*,**,+,x}$	14.00±1.37 $^{**, ++, xx}$	18.83±1.08 $^{*, **,+, x}$
Irreversibly changed	0.17±0.17	3.00±0.37 *	2.50±0.56 *	4.00±0.77 *	3.33±0.80 *	3.17±0.48 *
Degenerative forms	0.17±0.17	0.33±0.21	0.83±0.31	1.17±0.48	1.17±0.48	1.00±0.26 *

* $p<0,05$ in comparison with a group of almost healthy middle-aged people;

** $p<0,05$ in comparison with a group of almost healthy elderly people;

$^{+}$ $p<0,05$ in comparison with a group of elderly patients with benign prostatic hyperplasia;

$^{++}$ $p<0,05$ in comparison with a group of elderly patients with prostate cancer;

^{x} $p<0,05$ in comparison with a group of elderly patients with kidney cyst;

^{xx} $p<0,05$ in comparison with a group of elderly patients with kidney cancer
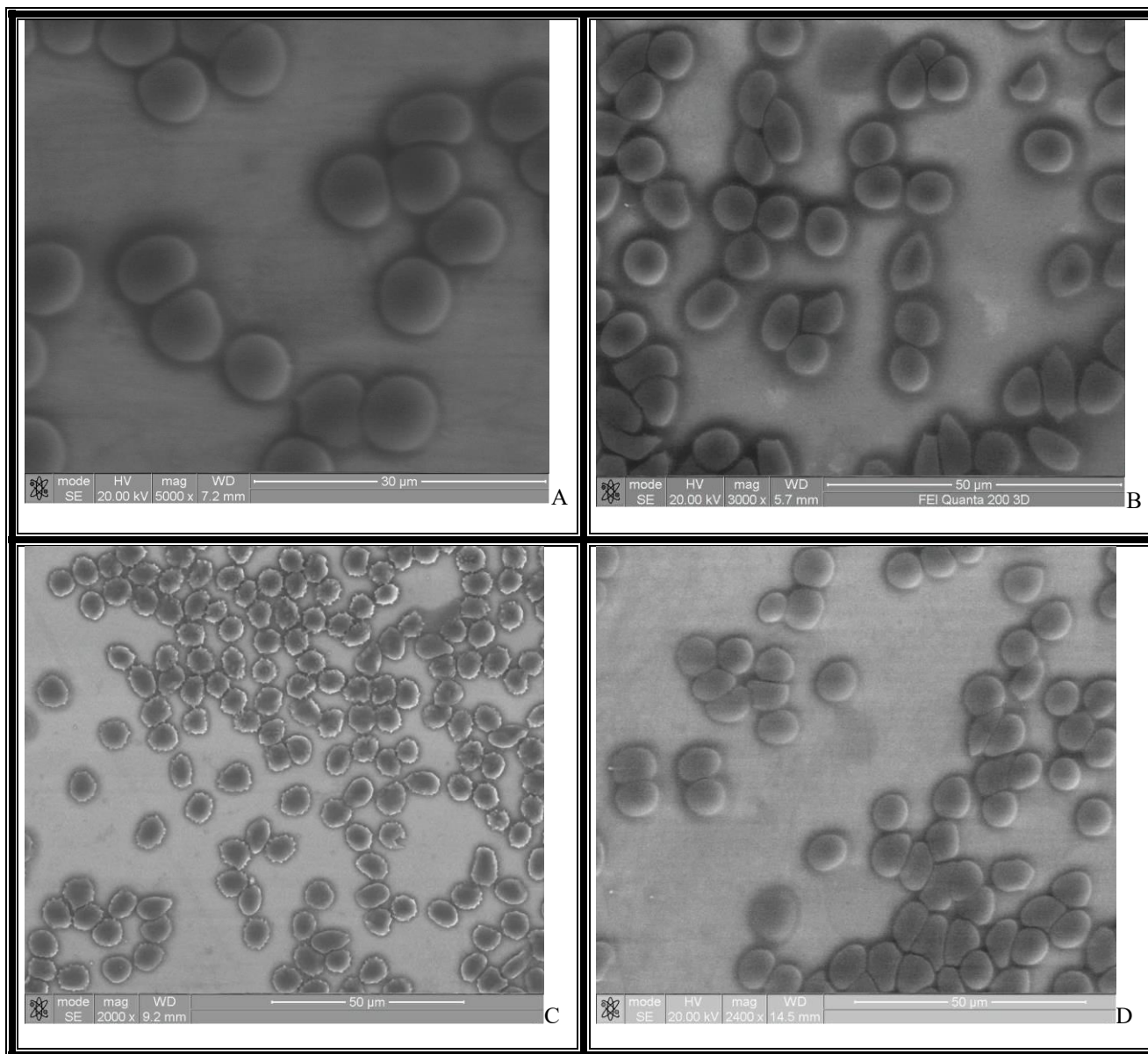
Figure 1 (A, B, C, D)

Fig. 1. A. Erythrocytes in a group of almost healthy people. SEM (x5000)

Fig. 1. B. Erythrocytes in a group with BPH. SEM (x3000)

Fig. 1. C. Erythrocytes in a group with prostate cancer. SEM (x2000)

Fig. 1. D. Erythrocytes in a group with kidney cyst. SEM (x2400)

The study of blood samples of patients with a kidney cyst revealed a morphological heterogeneity of the erythrocyte mass. The content of normocytes in the group was 91.9 ± 1.08%, which is reliably higher than this parameter in comparison with groups of patients with prostatic diseases (p <0.05). A reliable (p <0.05) decrease in the percentage of microcytes (0.4 ± 0.14%) was revealed in comparison with all studied groups. We also noted an increase in the macrocyte content: 7.8 ± 0.95%: p <0.05 compared to control groups of both ages, groups of patients with BPH, kidney cancer, and p> 0.05 relative to patients with prostate cancer. Cytometric data showed that the average size of red blood cells is 7.90 ± 0.05 μm. The diameter of the microcytes (6.4 ± 0.07 μm) is bigger than in all study groups (reliable (p <0.05) to groups of almost healthy people of both ages, patients with prostatic diseases). The diameter of normocytes (7.8 ± 0.04 μm) is bigger (p <0.05) than in all studied groups. The average size of macrocytes was 9.5 ± 0.08 μm, which is reliably higher than in the control groups of both ages and the group of patients with BPH.

The results of hemoscanning showed that in persons with diagnosed kidney cancer the main part of erythrocytes is represented by normocytes 93.3 ± 0.47%. At the same time, this indicator is higher among the study groups (reliably (p <0.05) in comparison with groups of patients with prostatic diseases). Wherein, the microcyte content (5.2 ± 0.34%) in the erythrocyte population was reliably lower in comparison with the control group and groups of patients with prostate diseases and higher relative to the group of patients with a kidney cyst (p <0.05). Macrocytes accounted for 1.5 ± 0.27%. The diameter of macrocytes was 9.7 ± 0.19 μm, which is reliably (p <0.05) higher, relative to the group of patients with BPH. In this group, the mean size of red blood cells was 7.40 ± 0.04 μm. The diameter of microcytes was - 6.2 ± 0.05 μm (reliably (p <0.05) higher in comparison with the control group and groups of patients with prostate diseases, kidney cyst). The diameter of normocytes is 7.4 ± 0.03 μm, which is reliably (p <0.05) higher in comparison with control groups of both ages and the group of patients with BPH, and also reliably (p <0.05) lower relative to the group of patients with kidney cyst.

The distribution of erythrocytes in persons suffering from kidney cancer by main groups was such that the percentage of discocytes accounted for 77.00 ± 0.68%, which is reliably (p <0.05) lower in comparison with the control group and the group of patients with BPH. The content of transient forms of erythrocytes (18.83 ± 1.08%) is reliably higher than

that in groups of almost healthy patients of both ages, and the kidney cyst, and also lower relative to the group of patients with BPH (p <0.05). An increase in the content of transient groups of erythrocytes was due to a reliable (p <0.05) increase in the discocyte content with an outgrowth (4.33 ± 0.61%) compared to all studied groups, as well as ellipses of 2.33 ± 0.42% and discocytes with multiple outgrowths - 10.83 ± 0.79%. Relatively (p <0,05) higher in comparison with the group of almost healthy middle-aged people was the content of pre-hemolytic forms (3.17 ± 0.48%). In this group there were single dome-shaped red blood cells and erythrocytes in the form of a "deflated ball". The content of degenerative forms was 1.00 ± 0.26%, which is reliably (p <0.05) higher in comparison with the group of almost healthy middle-aged people.

CONCLUSION:

Thus, when comparing the results of erythrocyte studies in patients with urologic and oncological pathology, a number of characteristic changes were identified. In this way, normocytes predominated in the erythrocytic mass in all studied groups. The content of normocytes in the group of patients with kidney diseases was higher in comparison with patients suffering from prostate diseases. At the same time, in the elderly, the content of microcytes in the total erythrocyte mass was higher than in the control group of middle aged people. The number of microcytes in patients with kidney diseases is lower than in groups of almost healthy people of both ages and with the pathology of the prostate gland. For almost healthy elderly people, a reliable increase in the content of irreversibly altered forms of erythrocytes was characteristic. With BPH in the elderly, compared with almost healthy people of the same age group, there was a decrease in the number of discs and an increase in reversibly altered forms (due to a pronounced increase in the content of discs with multiple outgrowths). In the group of patients with diagnosed prostate cancer there was a decrease in the level of discocytes and an increase in the content of transitional forms. The change in the percentage ratio (as well as in BPH) was mainly due to an increase in the percentage of discs with multiple outgrowths.

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