



# Diagnostic analysis of toxic damage due to a dose of zoledronic acid

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## Abstract

**Introduction:** Comprehensive analysis is necessary to analyze the widespread use of bisphosphonate drugs in the treatment and prevention of various diseases of connective tissue structures. The relevance of the study depends on the impact they have on the patient's somatic condition, given the number of early and late complications diagnosed by domestic and foreign clinicians.

**Materials and methods:** The methodology used was developed on Stavropol Regional Clinical Hospital from 2010 to 2022. A randomized and observational study of the state of connective tissue structures was conducted in 186 patients who had a history of taking **zoledronic acid** and developed bisphosphonate osteonecrosis of the jawbone. At the diagnostic and treatment stages, patients were tested using laboratory methods to monitor amino acids in biological fluids (blood), as well as bone blocks extracted intraoperatively using highly effective gas-liquid chromatography on AAA 500.

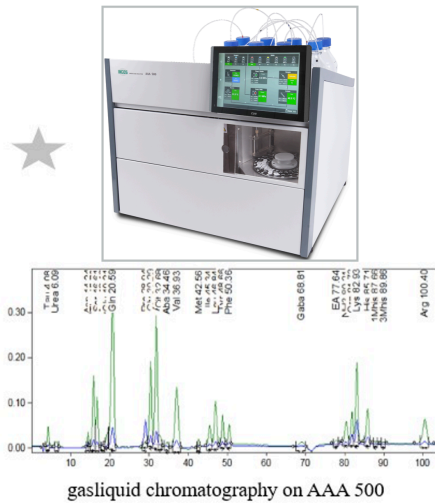
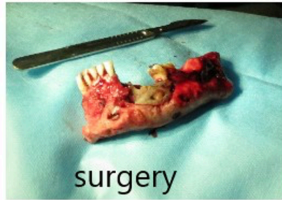
**Results and discussion:** Aminograms obtained in the pre-operative period indicate toxic effects of **zoledronic acid** on neuro, endocrine, and other human systems. The initiation and progression of aseptic inflammatory processes in tissue structures is correlated with the deteriorating general ocular status of patients when amino acids, such as **alanine**, **serine**, **taurine**, **phenylalanine**, **tyrosine**, **proline**, **histidine**, **glycine**, and **valine**, against a background of  $90.4 \pm 2.1$  mmol/L ammonia are reduced critically.

**Conclusion:** **Zoledronic acid** initiates an aseptic chronic inflammatory process in the jaw bones, accompanied by toxic acidosis, which worsens osteoporotic symptoms.

## Graphical Abstract

Hospitalization

Blood test



## Keywords

amino acids, highly effective gas-liquid chromatography, jawbone resection

## Introduction

Bisphosphonate preparations have been widely used by various clinicists for more than 15 years, particularly aminobisphosphonates such as **zoledronic acid** (Rodionova et al. 2022). Most of the current safety studies for **zoledronic acid** use included patients with osteoporosis of various etiologies, in which osteonecrosis of jawbone was detected in only 4.5 percent of patients per 10,000 (Kim 2021; Everts-Graber et al. 2022). A small percentage of this complication of anti-sickness therapy, according to a number of studies, does not exclude caution in the use of **zoledronic acid**, especially in patients with malignant pathology requiring an aggressive, versatile approach, and polyprogrammatic prescription of drugs.

The most commonly used drug is bisphosphonate that contains amines, and it has the highest affinity for hydroxylapatite and the most effective inhibition of farnesylpyrophosphatase. Intravenously administered, the drug binds to bone tissue at a percentage of 55-61%. According to the instructions for the preparation, the half-life of  $T_{1/2}$  is 0.24 h and 1.87 h, without affecting the functioning of the enzymatic systems of the liver and having minimal nephrotoxic action when following the instructions for the application and control of creatine clearance, which shall not exceed 35 mL/mL (Dhillon 2016). It is known that the introduction of **zoledronic acid** in a number of patients is accompanied by side reactions, both early and late, the most common being influenza-like syndrome, which includes hyperthermia, weakness, nausea, and other symptoms. Mechanism is due to the inhibition of farnesylpyrophosphate synthase, which stimulates the production of monocytes of isopentenel

pyrophosphate, resulting in the activation of gamma-delta CD14+ lymphocytes, which in the long term requires correction of treatment with bisphosphonic series drugs by the introduction of vitamin D3, to correct its insufficiency and drugs that eliminate hypocalcemia, arising in the same conditions (Dhillon 2016; Rodionova et al. 2022).

Global analysis of initiator routes Bisphosphonate osteonecrosis is still in progress, the main etiologic factor of its pathogenesis is surgical aggression (tooth extraction, osteotomy of exostoses, etc.), whereas there is no initial pathophysiologic link. As the first link in every metabolic reaction in the human body, the authors of this study were prompted to analyze amino acid indices. In addition to lipids, carbohydrates, and nucleic acids, amino acids are involved in all of the cellular life processes of the body. In a dynamic balance to support life processes, amino acids are found in the body in both protein-bound and free states. Free amino acids perform a specific produces of degradation products that are responsible for maintaining optimal pH balance (Golovanova and Tomashevsky 2019). Highly efficient gas-liquid chromatography is the most informative and fastest method for the determination of amino acids in connective-tissue structures, which was the choice in its favor in the present study.

## Materials and Methods

### Group description

At the Department of Neurosurgery of Stavropol Regional Clinical Hospital from 2010 to 2022, 186 patients were subjected to examination and combined

treatment. At the time of inclusion in the randomized, observational study, patients signed informed voluntary consents for participation in the study, photo-video recording of the clinical situation, use of specific laboratory methods of investigation, their interpretation and subsequent disclosure within the framework of scientific publishing activities, in particular the present article. Permission to conduct the present study No. 4 dated September 1, 2010, by the Ethical Committee on Stavropol Regional Clinical Hospital. The age of the patients – 88 men and 98 women – included in the study ranged from 32 to 68 years. All patients were diagnosed with bisphosphonate osteonecrosis of jaw bones of different extent and localization. The criteria for inclusion in the study were the presence of a history of **zoledronic acid** intake in the absence of active infusion and/or oral intake at the time of the study (no therapeutic or maintenance load), informed voluntary consent to participate in the study. The exclusion criteria were: defined as: absence of bisphosphonate osteonecrosis of jaw bones, somatic diseases in the decompensation stage, their exacerbation, and lack of consent for inclusion in the study. acute vascular disorders, exacerbation of general vascular pathology and underlying pathology, taking **zoledronic acid** preparations.

### Study design

For reliability, the analysis of indicators was performed at the inclusion stage, after the surgical stage (analysis of indicators in bone blocks). In addition to the patients' aminograms, a standard list of clinical and laboratory tests, including for such parameters as glucose and ammonia, was also studied. The indices were analyzed by taking peripheral blood and morning urine.

### Methods

The method of investigation was high-performance gas-liquid chromatography on an AAA 500 mass spectrometer (Ingos s.r.o., Czech Republic). Before entering the spectrometer, blood was centrifuged to isolate plasma to be analyzed. Plasma was then chemically deproteinized. Urine as well as blood was deproteinized. The amino acids studied are as follows: **alanine**, **L-arginine**, **valine**, **glycine**, **histidine**, **lysine**, **proline**, **serine**, **taurine**, **phenylalanine**, and **tyrosine**. The study of the mechanisms of initiation of metabolic acidosis determined the authors' choice of amino acid spectrum. The presented clinical picture, as well as a number of standard diagnostic methods in connection with the knowledge obtained from domestic and foreign literature sources, allowed assuming a quantitative change and further laboratory confirmation of it.

### Statistical analysis

The results were expressed as  $(M \pm m)$  and  $M(s)$ . The percentage of the confidence interval was equal to 95%. The Student's t-test was used for the comparison of the means between different groups, and within the same group in dynamics by using the ANOVA test of paired samples. Levels of significant ( $p < 0.05$ ) and reliable ( $p < 0.001$ ) differences in quantitative characteristics were assessed using non-parametric Mann-Whitney and Kruskal-Wallis criteria.

The level of statistical significance of the differences between the indicators was set at  $p < 0.05$ .

## Results and Discussion

At the time of preoperative examination, the biochemical analysis indices of interest to the authors such as markers of toxic analysis were as follows: glucose –  $8.2 \pm 2.4$  mmol/L and ammonia  $90.4 \pm 6.2$  mmol/L. Clinical evidence of 1 type diabetes prior to the use of **zoledronic acid** in patients had not been established by allied health professionals. The correlation of these indices associate with the severity of the general condition, with 30% characterized as moderate severity (56 patients) and 70% as severe severity (130 patients). The severity of the general condition was determined by general clinical and laboratory analysis and the quality of life criteria according to the MOS-36 questionnaire. Clinically, 65% of patients had functional fistulas (120 patients), and all the patients reported dysphagia, dysphonia, apathy, pain associated with limited mouth opening, and social discomfort. Critical reductions in the parameters of the amino acids of interest (Table 1) were detected by high-efficiency gas-liquid chromatography.

According to Loktionova et al. (2022), the alkalization of blood plasma by the release of phosphate into the interstitial space provided a cascade chain of biochemical disorders leading to the depletion of cellular reserves, in particular for a number of amino acids; in addition, phosphate leaching from bone tissue increased the degree of osteomalacia and, as a consequence, could initiate bisphosphonate osteonecrosis. **Alanine** deficiency contributes to a decrease in other amino acids. It synthesizes **leucine**, **isoleucine**, **valine**, and **serine**. In the patients tested, **alanine** was reduced 49-fold to 3.95 mmol/L, which was probably the trigger for excessive protein catabolism, exacerbating plastic and regenerative processes. The identified **L-arginine** deficiency was the cause of atherosclerosis, hypertension and thrombus formation due to a gradual increase in vascular tone and impaired blood coagulation, which was clinically confirmed in the study. Such pathological processes led to the development of local ischemia in the maxillofacial region, which is one of the factors of bisphosphonate osteonecrosis. Also, impaired biosynthetic processes decreased the values of **glycine** from 194.2 to 1.61 mmol/L, which was found in significant amounts in collagen, has a function in the construction of proteins and participates in ammonia neutralization. The structure of the connective tissue has been negatively affected by the degenerative processes. **Histidine** deficiency had a negative effect on tissue regeneration, and also contributed to a decrease in the level of newly formed hemoglobin, which correlated with laboratory indicators -HbA1c (glycated Hb)- 4.9%. The determined **serine** deficiency (1.63 mmol/L) contributed to the loss of the neurotransmitter acetylcholine, which legitimately impaired the transmission of peripheral nerve impulses to the brain as well. The **serine** deficiency identified (1.63 mmol/L) contributed to the loss of the neurotransmitter acetylcholine, which, of course, also impaired the transmission of peripheral nerve impulses to the brain. Probably, the noted neuropathophysiological processes contributed to a decrease in the secretory activity of salivary glands, which correlated with clinical data in 36% of patients. This factor also potentiated the development of a contractile dysfunction of the masticatory muscles, which occurred in 88% of the patients to a greater or lesser degree of severity.

The development of secondary hypoparathyroidism was determined by a decrease in [phenylalanine](#), a [tyrosine](#) precursor, and was clinically characterized by the development of hypercalcemia requiring drug correction. The mechanism of formation of pathological links of bisphosphonate osteonecrosis that led to disruption of the reparative cycle of bone is still not completely clear. The complex of conducted studies showed that plastic and regenerative processes in 82% of the examined patients were slow, and in 18% they were absent at all, against the

background of limited or disturbed local metabolism in tissues, first of all, in periosteum, with the predominance of acidosis. In the area of ischemia, accumulation of lactic and pyruvic acids with predominance of glycosaminoglycan conversion of carbohydrates was detected, which was most likely the cause of osteogenesis inhibition.

Postoperatively, amino acid levels were evaluated in bone blocks taken during jaw resection (Table 2).

**Table 1.** Quantitative amino acid profile of study patients in the preoperative

Quantitative indicator, (mmol/L)	Amino acid										
	Alanine	L-arginine	Valine	Glycine	Histidine	Lysine	Proline	Serine	Taurine	Phenylalanine	Tyrosine
Reference values	188.3–624.2	7.0–111.0	92.0–313.0	99.7–383.9	57.0–114.0	120.0–318.0	90.0–226.7	69.0–170.50	26.3–84.8	29.5–92.0	26.3–84.8
Pre-operative period	3.95±0.42	2.1±0.05	4.1±0.1	1.61±0.04	1.45±0.04	3.26±0.05	2.92±0.2	1.63±0.05	1.61±0.05	0.87±0.02	1.26±0.1
ANOVA test, p<0.05											

**Table 2.** Quantitative composition of amino acids in bone blocks (%)

Name of indicators	Regulatory document on test methods	Permitted levels	Test results, %	
			in absolute dry matter, %	in the original substance, %
Asparagine		-	2.58±0.05	1.60±0.5
Threonine		-	1.00±0.01	0.62±0.02
Serine		-	1.30±0.02	0.80±0.05
Glutamine		-	4.25±0.1	2.63±0.1
Proline		-	3.30±0.4	2.04±0.1
Glutamate		-	4.39±0.12	2.71±0.1
Alanine		-	3.18±0.05	1.96±0.1
Valine		-	1.54±0.01	0.95±0.05
Methionine	STATE STANDARD 32195 – 2013 (ISO 13903:2005)	-	0.43±0.01	0.27±0.01
Isoleucine		-	0.71±0.01	0.44±0.02
Leucine		-	1.92±0.05	1.19±0.1
Threonine		-	0.69±0.03	0.43±0.1
Phenylalanine		-	1.16±0.05	0.72±0.05
Histidine		-	0.72±0.02	0.44±0.05
Lysine		-	1.94±0.04	1.20±0.04
Arginine		-	2.41±0.1	1.49±0.1
Amount of amino acids		-	31.54±0.2	19.49±0.1
Total humidity		-	-	38.21±0.2
ANOVA test, p<0.05				

The results indicated a deficiency of **alanine**. This resulted in an increased need for branched-chain amino acids (**isoleucine**, **leucine**, **valine**) in the body. These data are an indication that there was a slowdown in the development of regeneration processes and their incompleteness. Thin, nonhealing oral mucosa is more easily damaged by chewing (Ruggiero et al. 2022; Abisalova et al. 2023; Sletov et al. 2023). Such conditions facilitated microflora penetration and increased the likelihood of bisphosphonate osteonecrosis, which was clinically confirmed in the present study. The deficiency of **isoleucine** and **leucine**, in turn, was reflected in the loss of muscle mass, as it has been proved that it has an important role in obtaining energy from muscle glycogen, and its deficiency led to the manifestation of hypoglycemia (Anastasilakis et al. 2022; Gandylyan et al. 2023). The identified **arginine** deficiency contributed to endothelial dysfunction in vascular wall function (Shcheblykin et al. 2022). A number of morphological studies revealed significant lipid deposits in the arterial intima, which was clinically manifested by increased blood viscosity with signs of progressively developing atherosclerosis. As a result, reparative bone regeneration was impaired due to excessive accumulation of lactic and pyruvic acids, glycosaminoglycans, and deficient blood supply, leading to impaired periosteal metabolism, which, in turn, contributed to the inhibition of normal osteogenesis (Loktionova et al. 2022; Sogacheva et al. 2022; Martins et al. 2023).

A comparative aspect with the currently existing studies in the field of analysis of the content of amino acids could not be carried out due to their absence in the Russian and foreign literature sources (Polyakov et al.

2016; Amin et al. 2024; Fernandes et al. 2024). At the same time, the histologic characterization performed by numerous clinicians is consistent with the picture of bisphosphonate-induced osteonecrosis of the jaw with prevailing suppression of osteogenesis and increased cell-free mass in the resected block obtained by the Loktionova et al. (2022).

## Conclusion

The toxic effect of **zoledronic acid** on the human body has been recorded by a number of researchers, while the mechanism of its initiation remains incompletely understood.

Influence on amino acid profile, actually one of the key links of practically any biochemical reaction, requires due attention and study, for the possibility of controlling early and late complications of therapy with the above-mentioned drug, which has positive dynamics of use in the treatment of the main pathology.

### Conflict of interest

The authors have declared that no competing interests exist.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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