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FREE AMINO ACIDS AS METABOLIC MARKERS*

SLOBODNE AMINOKISELINE KAO METABOLIČKI MARKERI*

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Abstract

Amino acids are basic materials for the life, playing various important roles in living systems. Free amino acids represent amino acids that are not bonded into the complex molecules, such as proteins or peptide. It has been documented that they are responsible for the proper metabolism in an organism. The present paper summarizes current opinions about roles of amino acids in overall metabolism, indicating possibilities to use free amino acid analysis as metabolic indicators. It is known that free amino acids participate in protein synthesis, they have regulatory roles of gene expression, proteolysis, hormone secretion, signal transduction, cell hydration and can be used as energy fuel under certain conditions (injury, sepsis etc). Recent finding indicate that free amino acids present in biological fluids (plasma, cerebrospinal or tissue fluids) reflect alterations of glutamate-glutamine-citrulline- arginine pathway, glucose-alanine cycle and other pathways. Qualitative and quantitative characteristics of free amino acids present in biological fluids can be used as good indicators of anabolic/catabolic, energy and nutritional status in an organism both in physiological and pathological conditions. Analysis of individual amino acids are widely used in defining metabolic status in various conditions, particularly in injury, sepsis, wound healing, liver failure, renal failure, organ transplantation etc.

INTRODUCTION

Amino acids are one of the basic materials for the life [1] playing many important roles in living systems. Although more than two centuries has past since the first amino acid was discovered (glycine 1806 year), these small molecules are still in focus of many investigations, continuously bringing new data and never stopping with surprises [2].

It is mainly due to continuous development of methods for their analysis. Namely, within the last century various methodological procedures were developed for amino acid measurement in biological fluids. They usually employ gas, liquid, or ion exchange chromatography, permitting separation of up to 35-40 different amino acids, while high-pressure liquid chromatography provides identification of even larger number of these compounds [3-5]. Since capillary electrophoresis was introduced as a new technology offering rapid separation of various ionic and/or ionizable compounds with low sample and solvent consumption, there were attempts to use it for amino acid studies [6-8].

DISTRIBUTIONS AND CLASSIFICATION OF AMINO ACIDS

In an organism amino acids are present as bounded, in peptide and protein molecules, and as free, i.e. unbounded ones. Only L-configuration of amino acids is physiologically active, and in this paper only L-configuration of all amino acids is considered. The greater quantities of amino acids are bounded, representing approximatively 99% of total nitrogen present in an organism. Free amino acids are constant constituents of all biological fluids (cells, blood, plasma, cerebrospinal fluid), but are present at very low concentrations [9-12]. Regardless their low quantities, free amino acids are important both as basic substrates and as regulators in many metabolic pathways [9, 13-15]. Also it has to be considered that their intracellular levels are greater comparing to the extracellular fluids [16].

According to the transport RNA (tRNA), amino acids can be classified to proteinogenic and non-proteinogenic (Figure 1). Proteinogenic amino acids have their own tRNA,

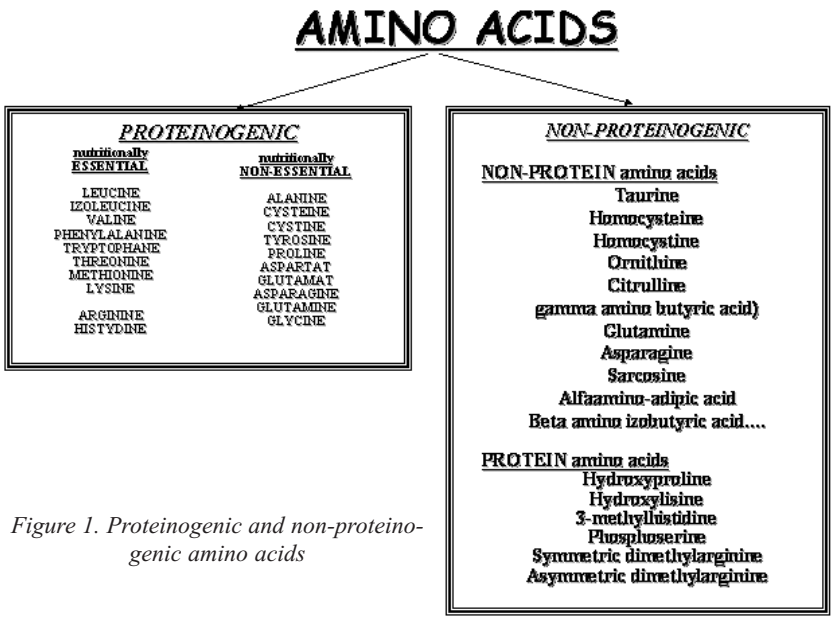

Figure 1. Proteinogenic and non-proteinogenic amino acids

comprise only 20 constitute monomer units of proteins and are divided into nutritionally essential (can not be synthesized in adequate quantities in an organism and must be taken from the exogenous sources) and nutritionally nonessential (can be synthesized from amphibolic intermediates). For human, phenylalanine, valine, leucine, isoleucine, methionine, threonine, lysine, and triptophane are essential, while arginine and histidine are semi-essential amino acids, as they can be synthesized in an organism, although not always in sufficient quantities. A human organism can synthesize alanine, glutamic and aspartic acid, tyrosine, glutamine and the other nonessential amino acids (Figure 1).

It has to be pointed out that some of the protein bounded amino acid can be transformed by posttranslational modifications either in physiological or pathological conditions, creating new amino acids such are 3-methylhistidine, symmetric and asymmetric-dimethylarginine, hydroxylysine, hydroxyproline, nitrotyrosine, gamma-carboxyglutamate etc, known as non-proteinogenic protein amino acids. There are also amino acids that can not be found in proteins, known as non-proteinogenic non-protein amino acids. They represent metabolic intermediates and substrates, comprising taurine, homocysteine, homocystine, citrulline, ornithine, gamma amino butyric acid, for example (Figure 1).

Both proteinogenic and non-proteinogenic amino acids can be found in free amino acid pools of various biological fluids. Their quantifications in biological fluids and tissues provide us important biochemical and nutritional information that enables the diagnosis of various diseases, especially metabolic deficiencies [12-13, 17-20].

ROLES OF FREE AMINO ACIDS

Free amino acids are important substrates for: protein synthesis; glucose and urea synthesis; energy production; synthesis of biologically active substances (e.g. nitric oxide, catecholamines, and thyroid hormones), creatinine and carnitine [21]. Transports of amino acids into the cells are of particular importance for their action [22]. Also, recent findings have indicate amino acids as important regulators of metabolism effecting proteolysis [23] enzyme activities [24-25], hormone secretion [26], gene expression [27-28], and cell hydration [29-30].

Regardless their small quantities, free amino acids have important role in overall metabolism in an organism, not only as substances involved in protein synthesis, but also as precursor in gluconeogenesis and as intra- and inter-cellular regulators and modulators of metabolic pathways. Free amino acids have various important metabolic processes including protein synthesis, regulations of gene expression and proteolysis, cell hydration, signal transduction, hormone secretion, too.

Protein synthesis represents the most important role of amino acids. It is well known that amino acids serve as precursors in protein synthesis [31]. A multitude of different proteins can be formed from only 20 common amino acids because they can be linked together in an enormous variety of sequences determined by the genetic code. However, branched-chain amino acid leucine may be particularly important as a key metabolic regulator of protein synthesis [32]. Also it has been shown that ageing muscle is less sensitive to lower doses of amino acids than the young and may require higher quantities of protein to acutely stimulate equivalent muscle protein synthesis [33].

Amino acids as regulators of gene expression have been undoubtedly documented, too. Namely, controls of gene expression by amino acid availabilities have been well documented in prokaryotes and lower eukaryotes and recently in human [27]. Amino acids act through a number of signaling pathways and mechanisms to mediate control of gene expression at the level of messenger RNA translation including modulation of eIF2B activity, changes in eIF4F assembly and alterations of phosphorylation of rpS6 [28].

Amino acids as regulators of proteolysis have been also reported [23]. It has been documented that proteolysis and protein synthesis are major processes contributing to the body protein turnover. Although there are the huge varieties of proteases in body, only the authophagic-lysosomal pathways are responsible for bulk proteolysis, while the ubiquitin-proteasome pathway plays a significant role in the fine control of the degradation of specific proteins. The data obtained with liver suggest that leucine, phenylalanine and tyrosine, in combination with a few other amino acids such as alanine and glutamine, are the most important amino acids involved in the control of authophagic proteolysis [34]. Amino acids, in combination with hormones, are well known to be primary regulators of body protein turnover [35]. Also, several amino acids have direct regulatory potential such are: leucine, glutamine, tyrosine, phenylalanine, proline, methionine, tryptophane and histidine in the liver, and leucine in the skeletal muscle authophagic proteolysis [23].

Cell hydration and changes in cell volume are additional roles of free amino acids. A new avenue of metabolic regulation by amino acids in mammalian cells was opened more than a decade ago with discovery that an increase in cell volume was associated with glutamine levels [29-30].

Amino acids are involved in signal transduction and hormone secretion, too. Relationship between free amino

acid pool alterations, protein synthesis and signal transduction have been documented during the last decades. It has been shown that leucine (together with phenylalanine and tyrosine) is most potent in inhibiting autophagy (34), while leucine is also an inhibitor of the lysosomal proton pump (36). In addition, branched-chain amino acids, comprising valine, leucine and isoleucine, stimulate insulin secretion [37], while arginine effects upon growth hormone secretion [38].

Also it has been documented that aromatic amino acids, particularly tryptophane and tyrosine have important roles in brain functions [39]. Tryptophan is an amino acid that brain converts into serotonin, a neurotransmitter that communicates messages between nerve cells and affects mood, while tyrosine is the precursor for synthesis of the catecholamines, dopamine and norepinephrine. It was confirmed that tryptophan uses the same transport systems in both fibroblasts and at the blood brain barrier [22]. In addition, disturbed transport of tyrosine, as well as other amino acids, across the blood-braine barrier has been found in a number of psychiatric disorders, such as schizophrenia, bipolar disorder and autism as well as in attention-deficit /hyperactivity disorder [22]. In the mammalian central nervous system, gamma-amino butiryc acid and glutamate are the major transmitters mediating inhibitory and excitatory synaptic events, respectively [40].

Amino acids modulate lipid and glucose metabolic pathways, too. Recent studies have shown that increasing intake of branched-chain amino acids have significant impact on lipid and glucose metabolism [41-43]. However, some studies have shown that increased branched-chain amino acids prevent high-fat diet-induced obesity [44-45], while the other observed that they have no effect or leads to insulin resistance [46]. Experimental studies have shown that mice fed a leucine-deficient diet for 7 days exhibit significant changes in lipid metabolism as demonstrate by suppressed lipogenesis in the liver associated with increased lipolysis in white adipose tissue, while isoleucine or valine deprivation stimulates fat loss via increasing energy expenditure and regulating lipid metabolism in this tissue [47]. It has been also shown that isoleucine supplement could prevent the tissue triglycerides accumulation [45] and balance blood glucose [48].

Amino acids as immunonutrients have important impact on immunity and whole body metabolism in various conditions. Even though immunonutrition has not been widely assimilated by clinicians other than nutritionists, immunonutrients including glutamine may exert beneficial influence on diverse patient populations [49]. Some data suggest that giving glutamine and arginine may be beneficial, in particular on inflammatory response and defense against pathogens, although future studies are needed in specific pathophysiological conditions [50]. Considering these findings there are more or less successful attempts to use amino acids in therapy of various diseases. The use of glutamine-enriched total parenteral nutrition might significantly decrease both the unwanted inflammatory reaction and the infectious morbidity, as well as improved nutritional status in postoperative gastrointestinal cancer patients [51].

INTERORGAN COOPERATION OF AMINO ACID METABOLISM

In last instance, all amino acids present in an organism originate from the food proteins. However, the availability of amino acids for their specific purposes is determined by

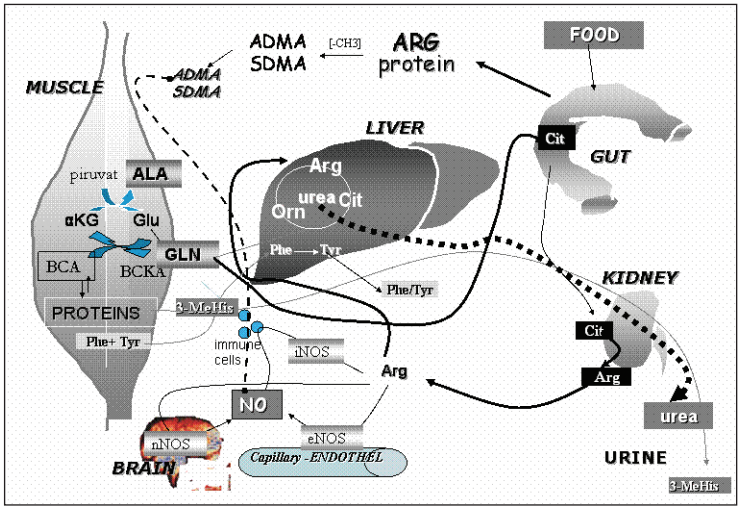

Figure 2. Interorgan cooperation of amino acid metabolism

the rate at which they disappear through conversion to other amino acids, breakdown, excretion and incorporation into proteins. Thus their plasma levels provide important information about various metabolic processes, although the actual flux of individual amino acids at the whole body level, across organs and within cells are much more important, but still needing investigations and resolving.

It has been shown that in metabolism of various amino acids interorgan cooperation occurs. The liver, skeletal muscles and kidneys have key roles, while vascular endothelium and enterocytes have been recognized as important sites in general metabolism of some amino acids, recently (Figure 2). The liver plays a central role in whole body amino acid metabolism, including protein synthesis and breakdown as well as several detoxification processes, notably those of end-products of intestinal metabolism, like ammonia [52].

It is known that free aromatic amino acids, such as phenylalanine and tyrosine are catabolized in liver as only this organ possess phenylalanine hydroxylase, an enzyme that converts phenylalanine to tyrosine [53]. On the other hand, branched-chain amino acids, accounting for approximatively 35% of the essential amino acids in muscle proteins, can be oxidized only in skeletal muscles [54-55]. Recently it has been documented that branched-chain amino acids represents regulators of insulin signaling [26]. Also it has been shown that exercise greatly increases energy expenditure and promotes oxidation of these amino acids and is believed that they contribute to energy metabolism during exercise as energy sources and substrates to expand the pool of citric acid-cycle intermediates and for gluconeogenesis. Thus, branched-chain amino acids supplementation has beneficial effects for decreasing exercise-induced muscle damages and promotes muscle-protein synthesis [55]. The others have shown that only leucine among branched-chain amino acids promotes muscle-protein syntheses in vivo, particularly when orally administered [56].

On the other hand, glutamine and alanine (Figure 2) are the main nontoxic transporting forms of ammonia from

peripheral tissues to liver, representing good indicators of ammonia metabolism in the peripheral tissues [57] and represent the most abundant amino acids in plasma and skeletal muscle free amino acid pools under physiological conditions. During metabolic acidosis, liver detoxification of ammonia is switched from urea synthesis to net production of glutamine which can be extracted from the circulation by the kidney to produce ammonium ion which serves to neutralize urinary acids [58].

In addition, glutamine and alanine are starting materials in various biosynthetic pathways participating as a carbon or nitrogen sources. Thus, alanine represent an important compound in glucose-alanine cycle [14], while glutamine represents precursors in production of purines and pyrimidines for synthesis of nucleic acids [59]. Also the carbon skeleton of glutamine may be utilized as a fuel by rapidly dividing cells such as enterocytes, lymphocytes and fibroblasts [60]. Thus, glutamine may have a vital role in the maintenance of intestinal integrity and function.

It has been also documented that enterocytes transform glutamine into citrulline, a non-essential amino acids that could serve as a substrate for renal arginine production 61). Recently it has been suggested that glutamine is a low-risk and low-cost therapeutic intervention that could protect cells and tissues against injury, attenuate inflammation, preserve metabolic function and may be an ideal intervention in the prevention/treatment of multiple organ dysfunction syndrome following sepsis or other injuries 62). Arginine, a nutritionally semi-essential, that can be synthesized in the kidney from citrulline, is involved in pathways producing various compounds having enormous biological importance, such as nitric oxide, polyamines, proline, glutamate, creatine, and agmatine [63].

FREE AMINO ACIDS POOL CHARACTERISTICS AS METABOLIC INDICATORS

Free amino acid pool qualitatively and quantitatively represents free amino acids present in a biofluids (blood, plasma, tissue, cerebrospinal fluid), considering existence of dynamic balance caused by their metabolic alterations. It has been documented that characteristics of free amino acids pool are good indicator of anabolic / catabolic, energy or nutritional status of an organism, both in physiological and pathophysiological conditions such as exercises, injury, sepsis, wound healing, transplantation etc [10-13, 6-17. 19-20]. Specific abnormalities in amino acid concentrations, associated with physiological conditions, have been reported in the context of various diseases, including liver failure, renal failure, diabetes, cancer, and other diseases [13, 17-18, 64-65]. On the other hand, some characteristics of free amino acids pool can be used as metabolic indicators, too.

Amino acids as indicators of protein metabolism

Proteolysis along with protein synthesis is a major process that contributes to body protein turnover and tissue development, and a slight decrease in synthesis or slight increase in degradation rates, if sustained, can result in a marked loss of mass in the organism as a whole. Thus, evaluation of protein catabolism in an organism is very important, particularly in catabolic states, including sepsis, cancer, burn injury, diabetes and other conditions.

Specific metabolic characteristic of phenylalanine and tyrosine make them useful as markers of whole body protein metabolism [53]. Thus the levels of phenylalanine and tyrosine, as well as their molar ratio, represent good indicators of net protein catabolism in peripheral tissues both in physiological and pathological conditions [10, 16]. They are also used as indicators of acute liver failure [66].

On the other hand alterations of 3-methylhistidine, both in plasma and urine, is an specific indicator of myofibrillar proteins catabolism as this amino acid is specific constituent of actine and myosin, which is formed by post-translational methylation of peptide bounded histidine [4, 67].

Along with 3-methylhistidine as a normal constituent of myofibrillar proteins, there are amino acids that can be formed only under pathological conditions. For example, nitrotyrosine represents a pathological amino acid, formed by nitration of peptide bounded tyrosine, due to the prolonged presence of high concentrations of nitric oxides in a region and/or in a whole organism [68]. Tyrosine nitration is usually considered to be an indication of high oxidative and nitrative stress that results in proteins damage. Nitrotyrosine adducts are formed in vivo by two reactions including the reaction of nitric oxide with superoxide leading to peroxynitrite production and the reaction of nitrite and hydrogen peroxide with various heme peroxidases. These reactions lead to formation of a tyrosyl radical which with nitrogen oxide yield 3-nitrotyrosine [68]. Following breakdown of the proteins in which tyrosine residue is nitrated, nitrotyrosine can be detected both in tissues, as well as in circulating free amino acid pool. Thus, nitrotyrosine represent an early indicator of inflammation.

Sulfur-containing amino acids as indicators of protein and amino acid metabolism

Sulfur-containing free amino acids, such as cysteine or homocysteine, have important roles in various metabolic conditions [69-70]. Homocysteine is a sulfur-containing amino acid formed from methionine during transmethylation reactions (70) which is either salvage to methionine by remethylation or is condensed with serine to form cystathionine, which is further catabolized to cysteine. Intracellular homocysteine is a product of S-adenosylmethionine-dependent transmetylation reactions, and it is either degraded or remethylated to methionine, while plasma homocysteine reflects the balance between the intracellular formation and utilization of this sulfur compound [69]. Also, their oxidation and/or interactions produce amino acids with disulfide bonds, such as homocystine, cystine or miffed disulfides homocysteine-cysteine. Recent findings indicate increased plasma homocysteine level as a good indicator of atherosclerotic risk [71], which also appears to be an independent predictor of heart failure [72]. Thus, the levels of thiol containing amino acids present in the physiological fluids could be important in defining metabolic status of an organism and there are increasing interests for their measurements [8].

Branched-chain amino acids as metabolic indicators

Circulating levels of valine, leucine and isoleucine, known as branched-chain amino acids, are catabolized in the peripheral tissues and represent regulators of insulin signaling, while their plasma levels reflects alterations in protein metabolism dependent on insulin action [26]. Also it has been documented that branched-chain amino acids are good markers of nutritional and energy status of an organism [73]. Namely, it has documented that plasma molar ratio between glycine and branched-chain amino acids consistently reflect protein intake, while plasma molar ratio between alanine and branched-chain amino acids reflects calorie intake [73]. Thus, plasma molar ratios between glycine to branched-chain amino acids and alanine to branched-chain amino acids reflect nutritional status of an organism [73]. From experimental and clinical data from the literature and their own investigations they tentatively assigned areas of nutritional significance to the ratiogram. However, future search for biomarkers of branched-chain amino acids status, utilizing proteomics and metabolomics, is needed [74].

On the other hand, it was observed that during renal failure, abnormalities of these amino acids are due to both the lack of renal contribution to amino acid metabolism and the impact of renal failure and acidosis on whole nitrogen metabolism (75). Low circulating levels of branched-chain amino acids and elevated of aromatic amino acids and methionine are hallmark of liver disease [76]. Supplementation of branched-chain amino acids appeared to be associated with decreased frequency of complications of cirrhosis and improved nutritional status in patients with liver failure [76].

Amino acids as indicators of nitric oxide metabolism

Nitric oxide (NO) is a ubiquitous intercellular messenger enzymatically synthesized from arginine by different isoforms of NO synthases. It is short lived molecule with half life of approximatively 5 seconds, which is metabolized to nitrites and nitrates, anion usually used as a measure of NO formation [77]. In low, a physiological concentration, NO is an important physiological messenger that modulates blood flow and neural activity, permitting both cell-cell communications and cytotoxicity. It is involved in various processes both in physiological and pathophysiological conditions [11-12, 78-79]. Importance of well coordinated arginine metabolism for proper NO formations have been recognized, too [11-12,79-80]. In addition, glutamate-glutamine-citrulline-arginine pathway and importance of inter-organ cooperation in regulation of arginine metabolism have been proposed, recently [81-82].

Also, it has to be pointed out that asymmetric dimethylarginine (ADMA) is the potent inhibitor of NO synthase and represents silent "uraemic toxin" as well as a global cardiovascular risk molecule [83]. It has been documented that ADMA represent a potent and long-lasting endogenous inhibitor of NO formation and is though to be a key player in the process of chronic vascular disease [83], while recently it has been proposed that ADMA might be a candidate of therapeutic target in gastric mucosa damage [84]. Thus, measurement of amino acids directly or indirectly involved in arginine metabolism, such as ADMA, citrulline, ornithine and glutamine are useful markers in various diseases.

In summary, qualitative and quantitative characteristics of free amino acids pool present in biological fluids are good indicator of anabolic/catabolic, energy and nutritional status of an organism both in physiological and pathological conditions. It reflects alterations of glutamate-glutamine-citrulline- arginine pathway and metabolism of individual amino acids and is widely used in various conditions, particularly in injury, sepsis, wound healing, liver failure, renal failure, and renal transplantation.

Apstrakt.

Aminokiseline predstavljaju osnovu života i imaju mnoge važne uloge u živim sistemima. Slobodne aminokiseline predstavljaju aminokiseline nevezane u kompleksne molekule kao što su proteini i peptidi. Pokazano je da su one odgovorne za pravilno odvijanje metaboličkih procesa u jednom organizmu. U ovom radu su sumirana savremena shvatanja o ulogama aminokiselina u celokupnom metabolizma i ukazuje se na mogućnosti korišćenja analize slobodnih aminokiselina kao metaboličkih indikatora. Poznato je da slobodne aminokiseline učestvuju u sintezi proteina, ispoljavaju regulatorne uloge u ekspresiji gena, proteolizi, sekreciji hormona, prenosu signala, hidrataciji ćelija, a u posebnim uslovima organizma (povreda, sepsa i drugo) u nedostatku drugih substrata mogu da se koriste za dobijanje energije. Najnoviji nalazi pokazuju da slobodne aminokiseline prisutne u fiziološkim tečnostima (plazma, cerebrospinalna ili tkivne tečnosti) odražavaju promene glutamat-glutamin-citrulin- arginin, glukoza-alaninskog i drugih metaboličkih puteva. Kvalitativne i kvantitativne karakteristike pula slobodnih aminokiselina prisutnih u biološkim tečnostima mogu da se koriste kao dobri indikatori anaboličkog/ kataboličkog, energetskog ili nutricionog statusa organizma i u fiziološkim i u patološkim uslovima organizma. Analiziranja individualnih aminokiselina se koriste za definisanje metaboličkog statusa u raznim uslovima organizma kao što su povreda, sepsa, zarastanje rane poremećaj funkcije jetre i bubrega, transplatacija bubrega itd.

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