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PEDIATRICS

INFECTION DISEASES

ANTIBIOTIC RESISTANCE: COMPREHENSION OF THE PROBLEM
(REVIEW)

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Abstract
Antibiotic resistance (also referred to as drug resistance) is one of the biggest public health challenges of our time. Bacterial infection has become a serious threat to life once again that brings about revision of costly and laborious processes of licensing and regulation of new antibiotics. Understanding the biochemical and genetic basis of resistance is of paramount importance to design strategies to curtail the emergence, spread of resistance, and devise innovative therapeutic approaches to multidrug-resistant organisms. Intrinsic antibiotic mechanisms are normally chromosome-encoded and include non-specific efflux pumps, antibiotic inactivating enzymes, or mechanisms that serve as permeability barriers. The acquired resistance mechanisms are generally obtained by horizontal gene transfer and include plasmid-encoded specific efflux pumps and enzymes that can modify the antibiotic or the target of the antibiotic. These mechanisms pose a more serious threat to human health because of a change in the context of the resistance determinant from chromosomal to plasmid-mediated, resulting in their enhanced expression and dissemination. The collection of resistance genes termed 'resistome' encompasses both intrinsic and acquired resistance genes. Tolerance that can result from mutations and from environmental conditions can occur in a subpopulation of phenotypic variants cells called "persistence", which is a specific type of tolerance. Increasing evidence suggests that tolerance and persistence play a considerable and currently underappreciated role in the recalcitrance and relapse of bacterial infections. The ability of many microbial species to grow as biofilm has further complicated the treatment of infections with conventional antibiotics. The mechanisms of antibiotic tolerance and resistance in biofilms have, in many cases, a genetic basis.
The novel approaches for tackling the antimicrobial resistance crisis must be part of global response to this problem such as phage therapy, antimicrobial peptides (AMPs), bioactive adjuvants, nano-biotechnology, an alternative approaches use gene-specific peptide (PNA), mesenchymal stromal cells, antivirulence therapies, and prophylactic vaccines. The application of specific genome engineering and synthetic biology (SB) methods such as recombineering, clustered regularly interspaced short palindromic repeats (CRISPR), and bacterial cell-cell signaling mechanisms for pathogen targeting are currently essential.
Scientific challenges encompass the discovery molecules with new chemical structures. Economic and scientific obstacles should be overcome by funding researches of advanced drugs and conceptual approaches.
Key words: *antibiotic resistance, bacteriophage, biofilm, nanotechnology, new potential antibacterial therapy, vaccination.*

Antibiotic resistance is one of the biggest public health challenges of our time and can also be referred to as antimicrobial resistance or drug resistance. Each year in the U.S., at least 2.8 million people get an antibiotic-resistant infection, and more than 35,000 people die [1]. Until the early 1980s, the pharmaceutical industry developed and introduced many new antibiotics to resolve the resistance issue, and with the passage of time, the pace of antibiotic development staggered, so very few new antibiotics were introduced. It brings about revision of costly and laborious processes of licensing and regulation of new antibiotics, and addresses the economics of antimicrobial drugs (cost of use vs. profit) [2]. Antibiotics are taken for only a short duration for most curable diseases by the patients as compared to drugs for chronic diseases such as heart disease or high-blood pressure, which is the

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reason for companies abandoning the antibiotic area [3].

Antimicrobial resistance (AMR) is a natural phenomenon whereby bacteria evolve in such a way to withstand the action of drugs, making them apparently ineffective. There are four mechanisms through which bacteria become resistance to antibiotics: 1) enzymatic inactivation (for example, beta-lactamase enzymes, which hydrolyze beta-lactams such as penicillins, cephalosporins); 2) drug extrusion by efflux pumps (proteins, which are able to extrude a wide variety of compounds (including antibiotics) out of the cell; 3) decreased uptake by changes in the outer membrane permeability or by presence of porins; 4) modification of the drug target (these changes impede the binding of the antibiotic and limit its potency) [4]. "Survival of the fittest" is a consequence of an immense genetic plasticity of bacterial pathogens that trigger specific responses that result in mutational adaptations, acquisition of genetic material or alteration of gene expression producing resistance to virtually all antibiotics currently available in clinical practice. Therefore, understanding the biochemical and genetic basis of resistance is of paramount importance to design strategies to curtail the emergence, spread of resistance, and devise innovative therapeutic approaches against multidrug-resistant organisms. In order to provide a comprehensive classification of the antibiotic resistance mechanisms, it is categorized them according to the biochemical and genetic routes involved in resistance. Biochemical reasons: i) modifications of the antimicrobial molecule, ii) prevention to reach the antibiotic target (by decreasing penetration or actively extruding the antimicrobial compound), iii) changes and/or bypass of target sites, and iv) resistance due to global cell adaptive processes. From an evolutionary perspective, bacteria use two major genetic strategies to adapt to the antibiotic "attack", i) mutations in gene(s) often associated with the mechanism of action of the compound, and ii) acquisition of foreign DNA coding for resistance determinants through horizontal gene transfer [5]. Resistance towards antibiotics is acquired by bacteria through either vertical evolution (endogenous) or horizontal evolution (exogenous). Vertical evolution involves the occurrence of a spontaneous mutation within the bacterial genome that confers on the bacterium (and subsequently its progeny) increased resistance to a given compound. Horizontal evolution involves the transfer of a resistance gene from a resistant bacterium to a susceptible bacterium. The mechanisms through which it can occur are conjugation, transduction and transformation [6]. Intrinsic antibiotic mechanisms are normally chromosome-encoded and include non-specific efflux pumps (which is likely evolved as a general response to environmental toxins), antibiotic inactivating enzymes, or mechanisms that serve as permeability barriers. A well-studied example of an intrinsic resistance system is the AcrAB/TolC efflux pump in Escherichia coli and vancomycin resistance in E. coli and other Gram-negative organisms. The acquired resistance mechanisms, on the other hand, are generally obtained by horizontal gene transfer (HGT) and include plasmid-encoded specific efflux pumps (such as TetK and TetL of *S. aureus*) and enzymes that can modify the antibiotic or the target of the antibiotic (mobilization of the chromosomal β-lactamase gene ampC to a plasmid resulting in its worldwide dissemination). These mechanisms pose a more serious threat to human health because of a change in the context of the resistance determinant from chromosomal to plasmid-mediated, resulting in their enhanced expression and dissemination [7].

The collection of resistance genes in a given environment, which is termed the 'resistome', encompasses both intrinsic and acquired resistance genes, as well as proto-resistance genes and silent or cryptic resistance genes. It is thought that many clinically relevant antibiotic resistance genes have their evolutionary origins in environmental microorganisms. Further evidence was provided by a recent functional screen of soil metagenomes, which revealed the presence of environmental antibiotic resistance genes that have >99% nucleotide identity to resistance genes in pathogenic isolates [8]. The ability of a whole bacterial population to survive longer treatments with bactericidal antibiotics is denoted as "tolerance". Tolerance that can result from mutations and from environmental conditions is able also to occur in a subpopulation of phenotypic variants cells called "persistence", which specific type of tolerance. Increasing evidence suggests that tolerance and persistence play a considerable and currently underappreciated role in the recalcitrance and relapse of bacterial infections. Tolerance and persistence may facilitate the development of resistance in a more intricate fashion. On the one hand, high levels of persistence or tolerance lead to a higher number of viable cells during antibiotic treatment, which results in an increased statistical probability for the occurrence of resistance-

conferring mutations. On the other hand, increased persistence, and possibly also tolerance, is pleiotropically linked with increased mutation rates both in growing cells (when antibiotic concentrations are low) and in persisters (when antibiotic concentrations are high). Understanding, detecting, and targeting tolerance and persistence will require joint efforts of microbiologists and clinicians and should eventually lead to reduced therapy failure [9]. Bacteria may adapt to unpredictable disturbances by increasing phenotypic heterogeneity, which can be produced by stochastic noise in gene expression. The variability in gene expression contributes to antibiotic tolerance, due to growth rate dependent killing. A certain frequency of nearly-dormant cells, so called persisters, is naturally produced by stochastic partitioning of proteins after cell division and represents an ancient evolutionary survival strategy, bet-hedging, that can help bacterial populations to survive antibiotic exposure. Phenotypic heterogeneity may thus be an adaptive strategy for the bacteria to cope with unpredictable antibiotic treatments, thereby rendering them inefficient [10].

The ability of many microbial species to grow as biofilm has further complicated the treatment of infections with conventional antibiotics. Indeed, microbial biofilms, that is microbial communities growing attached to abiotic surfaces (medical devices, surgical instruments, industrial pipelines, etc.) and tissues, are known to be an optimal environment to amplify both naturally occurring and induced antibiotic-resistance phenomena. That together with other defense mechanisms significantly increases biofilm antibiotic tolerance [11]. Cells of biofilm that turn to a nongrowing but still active state may quickly acquire tolerance to some agents. The dormant state is also metabolically inactive and nongrowing. To enter the dormant state, however, the cell has implemented protective modifications. Such modifications could include, hypothetically, alteration of membrane lipid and porin composition to reduce permeability, hibernation of ribosomes, inhibition of transcription and replication machinery, and deployment of enzymes that protect against oxidative stress without consuming ATP (e.g., catalase). Examples of stress responses that have been demonstrated in bacterial biofilms include catalase induction upon treatment with hydrogen peroxide, β-lactamase induction upon treatment with imipenem, and induction of the lipopolysaccharide-modifying pmr operon upon treatment with colistin. In each of these examples, the induced gene or genes enhance the capacity of the biofilm to tolerate the antimicrobial either by augmenting destruction of the antimicrobial agent or by modifying the cell to make it less susceptible [12]. It is important to acknowledge that, when in a biofilm, pathogenic bacteria employ both tolerance and resistance mechanisms to withstand antimicrobial challenges. It has also become clear that the underlying mechanisms of antibiotic tolerance and resistance in biofilms have, in many cases, a genetic basis. It is generally accepted that the basis for biofilm-specific antibiotic resistance and tolerance is multifactorial, and mechanisms of resistance and tolerance vary depending on the particular antimicrobial agent, the bacterial strain and species, the age and developmental stage of the biofilm, and the biofilm growth conditions [13]. Biofilms are known to control their population density through a cell-to-cell signaling mechanism known as quorum sensing. The benefit of quorum sensing is not limited to controlling population density. In fact, quorum sensing has also been shown to aid the spread of beneficial mutations throughout the biofilm colony, enhance access to nutrients, and contribute to antibiotic tolerance. Biofilms are recalcitrant to antibiotic therapy and a major cause of persistent and recurrent infections by clinically important pathogens worldwide (e.g., *Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus*). The same mechanisms (mutations, efflux pumps, and antibiotic modifying enzymes) do not appear to be the main cause of biofilm-mediated antibiotic tolerance. The extracellular matrix encasing the cells in a biofilm, also referred to as the Extracellular Polymeric Substance (EPS), is composed of a complex mixture of proteins, lipids, nucleic acids (extracellular-DNA), and polysaccharides. These constituents not only assist in securing the biofilm to the surface, but also trap nutrients, provide structural support, and shield against host immune responses and antimicrobial treatments. In addition, the EPS is also responsible for enabling cell-to-cell communication (quorum sensing), and facilitating the exchange of genetic material through horizontal gene transfer [14].

The antibiotic selection pressure is another factor of modifying the host's microbiota. The host system is subjected to a phenomenon of "selective pressure" when treated with antibiotics during infection. With a greater activity scale, the resistance frequency increases. This results in the resistant species surviving in the host population as compared to the susceptible strain with the harsh effect of the antibiotic. Thus, being a

reservoir increases the chances of infection spread to a greater extent. Antibiotics like cephalosporins, azithromycin, and fluoroquinolones bring about this effect of "selective pressure" in eukaryotic hosts. A careful and considerate use of antimicrobials is highly recommended for human safety [15]. However, it can be anticipated that antibiotic resistance will continue to develop more rapidly than new agents to treat these infections become available and a better understanding of the molecular, evolutionary and ecological mechanisms governing the spread of antibiotic resistance is needed [16].

The investigation of novel approaches for tackling the antimicrobial resistance crisis must be part of global response to this problem if an untimely reversion to the pre-penicillin era of medicine is to be avoided [17]. The advanced drugs as well as several conceptual approaches are an abundance of new and "old" revisited approaches being studied, which may move us from the end of the antibiotic era towards a new dawn of antibacterial agents. There has been a resurgent interest in the use of lytic bacteriophages to manage bacterial infections [18].

Antibiotics are expected to interfere with aspects of bacterial physiology that can be crucial to phage antibacterial activities, e.g., by interfering with bacterial ribosome functioning. Emphasis here therefore is on documenting the impact of antibiotics on phage-infection pharmacodynamics aspects, i.e., retention of phage ability to negatively impact targeted bacteria despite antibiotic co-treatment. These pharmacodynamic properties include a retention, by bacteria-infecting phages, of both gene expression and antibacterial activity, and also of associated in situ-within treated patients-phage virion production [19]. Now we need to pay more attention to innovatively recruit all phage resources for phage applications. For example, despite better-known lytic DNA phages, we should pay attention to temperate DNA phages, and even RNA phages, for bioplatforms. Given that temperate phages do not rapidly kill the host bacteria, instead actively modifying the properties and behaviors of them, they are likely armed with genetic resources which have evolved to actively manipulate the host's bacterial physiology and may provide clues concerning new antibacterial targets and proteins. As exemplified, was identified a phage protein (Tip) and its new antipathogenic target (PilB), from an unmodified temperate phage that displayed therapeutic efficacy in acute infections caused by *P. aeruginosa*. In that temperate phages' life cycles have focused on establishing a co-flourishing symbiosis with that of their host bacteria, they may be advantageous over lytic phages for reducing the emergence of antibacterial resistance. [19]. Many antibiotics (fluoroquinolones and β-lactams) can induce the expression of prophage gene products or lead to the excision and propagation of temperate phages activating and spreading hitchhiker prophages. This antibiotic effect may have unexpected outcomes on the virulence of bacterial populations and their resistance to antimicrobial drugs. These unwanted side-effects of antibiotics cast doubt on the suitability of some antimicrobial treatments and may require new strategies to prevent and limit the selection for virulence [18]. The very interaction of phages with the surface of bacterial cells may itself have an additive effect for phage therapy. It has been shown that bacteriophages, by attaching themselves to the bacterial surface at particular sites, could block resistance mechanisms such as an efflux pump or impair the fitness or the virulence factor of a bacterium. This would then make certain bacteria (i.e., *P. aeruginosa or K. pneumoniae*) more susceptible to traditional antibiotics and facilitate the healing of certain pathologies, such as endocarditis or vascular prosthesis infections [20].

The invaluable advantage of the specificity of phages is also a disadvantage: A suitable phage must be found/screened for an individual patient; a phage mixture could be an alternative in acute situations. The larger a stock collection of purified phages for a certain bacterial species is, the better the chance to find suitable phages [21]. The approach to phage therapy should be doubly effective; success is achieved when phage lyse the target bacterium, but also when bacteria evolve phage resistance because they suffer reduced virulence or increased sensitivity to antibiotics [22].

Bioengineering of phages could dramatically increase their therapeutic potential via a range of mechanisms, including expanded host range, switching host tropism, delivery of exogenous genes, or modification of phage capsids. The research was carried out for the host range of E. coli phage T2 by incorporating the long tail fiber genes of phage IP008 through homologous recombination. The resulting chimeric phage exhibited the broader host range of phage IP008, while still maintaining the strong lytic activity of phage T2. The engineered virus was capable of reducing biofilm cell counts by >100-fold compared to the wild-type phage. The genetic material that can be delivered or inserted into the

bacterial cell includes dominant sensitive genes to reverse antibiotic resistance, CRISPR/Cas9 sequences to inactivate virulence genes, modified lethal transcription regulators, small regulatory RNAs to silence antibiotic resistance determinants, and even genes that code for proteins capable of increasing the susceptibility to specific antibiotics [23]. Phage engineering technologies allow generating variants with unique properties and helping minimum the features that might hamper the applications of phage for prophylactic and therapeutic applications. All these methods are based on the homologous recombination. Alternatively, engineered phages can be directly generated by transforming the host cells with naked full-length phage genomic DNA containing the desired mutations [24]. Two slightly different medium-term strategies have been developed: the "magistral" phage production, introduced in Belgium recently, and the sustainable large-scale long-term access supply inventory, the latter requiring phage banks with substantial holdings of purified or pre-purified phages, both finally tailor-made flexible medicine. For true emergency applications with less common bacterial strains, compassionate use for individuals in hopeless situations should be possible. It seems crucial to include allowing immediate use of phages newly isolated with minimal regulatory requirements beyond those defining a complete production route. It is an additional advantage that phages and antibiotics are acting synergistically so that overnight phage screenings (phagograms) against patient isolates in parallel with antibiograms can produce results for tailored application [21].

Another approach considers the antimicrobial peptides (AMPs), which are predominantly small cationic peptides with hydrophobic regions containing between 10–100 amino acids, especially arginine residues, which allows them to interact with negatively-charged membranes, causing the direct destabilization of the surface of membranes with pore formation and subsequent cell lysis. Also, they have been described as chemotactic agents, modulating the immune system, and therefore constituting a bridge between innate immunity and adaptive immunity [25]. Although the principal antibacterial activity is attributed to their membrane-lytic mechanism, AMPs have also been demonstrated to function in host immune modulation, often by enhancing protective immunity and suppressing inflammation. They can influence processes like cytokine production, antigen presentation, chemotaxis or wound healing, complementing their bactericidal activity. For example, the AMP LL-37, found mainly in the granules of neutrophils, is an antisepsis agent that upregulates the production of interleukin-8 and leads to recruitment of leukocytes to the site of infection. Antimicrobial peptides and proteins with similar characteristics are very promising for the development of potential therapeutics to use against multiple antibiotic-resistant infectious diseases. Aside from the empowering effect in the immune system and their membrane-lytic abilities, AMPs can affect several bacterial processes such as macromolecular synthesis (i.e., RNA, DNA synthesis) protease inhibition, and protein-folding inhibition [26]. The most abundant synergy, including the interactions with human endogenous AMPs, is observed for antibiotics targeting protein biosynthesis, such as aminoglycosides and macrolides. Thus, these antibiotics may enhance the antimicrobial activity of host defensive molecules as well as can be used in combinations with AMP-derived antimicrobial drugs. The ability of AMPs to permeabilize bacterial membranes plays central role in their synergy with other antimicrobial compounds, but also indicates that this ability could be in turn modulated by the second substance in the combination contributing to the combined effect [27]. The diverse array of AMPs acts through different mechanism, and because many AMPs are bactericidal as opposed to bacteriostatic, it is unlikely that bacteria will be able to respond to these AMPs by adopting resistance strategies. Mesenchymal stromal cells (MSC) are adult multipotent progenitor cells, present in a variety of tissues and organs and contribute to healing processes by participating in the inflammatory, proliferative and remodeling phases of tissue repair. The present study is the first to show that equine MSC possess antimicrobial properties by inhibiting the growth of *E. coli* and *S. aureus*, in part by secreting antimicrobial peptides (AMPs) and depolarizing bacterial cell membranes. This antibacterial activity may contribute to the value of MSC as a therapy for chronic cutaneous wounds, where colonization by pathogenic bacteria commonly inhibits normal healing [28]. Accumulating evidence shows that in addition to acting at the cell membrane, AMPs may act on the cell wall, inhibit protein folding or enzyme activity, or act intracellularly in different mechanism of action on gram-negative and gram-positive bacteria. While AMPs should not cause widespread resistance due to their preferential attack on the cell membrane, in cases where specific protein targets are involved, the possibility exists for

genetic mutations and bacterial resistance. Indeed, the potential clinical use of AMPs has raised the concern that resistance to therapeutic AMPs could be associated with resistance to endogenous host-defense peptides. Current evidence suggests that this is a rare event that can be overcome by subtle structural modifications of an AMP [29].

Many AMPs exhibit direct microbial killing activity and also play an integral role in the innate immune system. These properties make AMPs attractive alternative antimicrobial agents. Furthermore, AMPs are promising candidates to be used as adjuvants in combination with current antibiotics in order to combat antibiotic resistance. Combinations of AMPs and antibiotics are less likely to develop resistance or transmit cross-resistance. The further identification and therapeutic development of AMPs and antibiotic-AMP combinations are strongly recommended [30]. Recent studies have shown that in particular cationic AMPs, such as LL-37, piperacillin, buforin II, ceprocin P1, indolicidin, nisin, and magainin II, are remarkably effective in combination with antibiotics like polymyxin E, piperacillin, azithromycin, daptomycin, linezolid, and clarithromycin to enhance antibiotic bioavailability against highly multidrug-resistant gram-negative and methicillin-resistant *S. aureus* (MRSA) pathogens. More than to enhance oral bioavailability, the strong membrane permeabilization capacity of AMPs (a novel synthetic cyclolipopeptide analog of polymyxin AMP38) can directly kill even dormant biofilm-forming cells in combination with classical antibiotics. An example demonstrating the efficacy of AMP-antibiotic therapy to remove biofilm is the treatment of *Pseudomonas aeruginosa* (*P. aeuruginosa*) with carbapenems [31].

The paradigm entails the use of bioactive adjuvants that augment the antibiotic efficacy of a primary antibiotic against drug-resistant pathogens. The adjuvant may possess weak to no antibacterial activity on its own but is able to either impede antibiotic resistance mechanisms or potentiate antibiotic action. An adjuvant may be an efflux pump inhibitor (EPI) (to prevent the extrusion of drugs), a membrane permeabilizer (to increase the number of molecules that penetrate the membrane), or an enzyme inhibitor (to prevent the degradation of drugs before they reach their targets). Clavulanic acid by itself possesses poor intrinsic activity against pathogens, but it efficiently inhibits widespread β-lactamases such as many types of the extended-spectrum β-lactamase (ESBL) family. The adjuvant aspergillomarasmine A (AMA) was recently discovered to resuscitate the biocidal activity of the carbapenem drug meropenem against metallo-β-lactamase-producing organisms [32]. One such approach is the use of adjuvants capable of revitalizing the activity of current existing antibiotics from resistant pathogens. Recently were reported a series of tobramycin (TOB)-based hybrid adjuvants that were able to potentiate multiple classes of legacy antibiotics (fluoroquinolones (moxifloxacin and ciprofloxacin), tetracyclines (minocycline), or rifamycin (rifampicin) against various multidrug-resistant (MDR) Gram-negative bacteria (GNB) [33].

However, despite the multiple beneficial properties of AMPs, they present some disadvantages such as: (I) degradation by proteases, both in the bloodstream and in the gastrointestinal system; (II their union with others proteins, which leads to their inactivation; (III) low metabolic stability and oral absorption; (IV) rapid excretion through kidneys and liver; (V) high toxicity and immunogenicity; and (VI) high production costs. For these reasons, their use for in vivo applications has not been fully satisfactory and only a few of them were explored in clinical trials. One of the main goals of nanotechnology is the design of nano-carriers, promising biomaterials that could increase therapy efficacy, minimize side-effects, and offer a controlled pharmacokinetic profile. Diverse types of organic nano-materials, including polymers (natural polymers such as DNA, cellulose, or chitosan, and many others, such as poly(lactide-co-glycolide) (PLGA) or PEG, are synthetic), liposomes, hydrogels, self-assembly systems formed by surfactants, (block co)polymers, and polar lipids polymer (micro)gels are utilized for study. A wide range of inorganic nanoparticles/ nano-materials, each offering system-specific opportunities, have been explored as delivery systems not only in the transport of peptides, but also for gene therapy, cancer treatment, and drug delivery such as gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), carbon nanotubes (CNTs) [34], ZnO, CuO and Fe2O3 [18]. It has been reported that peptides in nanostructures presented lower cytotoxicity, reduced degradation and increased efficiency at the desired target [35]. Nano-materials can be defined as the materials that have at least one dimension in the nano range (1–100 nm), whereas nanoparticles (NPs) are particles with at least one dimension in the nano range and can be as small as 0.2 nm. There are five features of nano-materials that make them a possible alternative to antibiotics. First, nano-

materials can easily penetrate the bacterial cell membrane and damage its structure, resulting in bacterial cell death. Second, suggested mechanisms of antibacterial activity of nano-materials are similar to the action of antibiotics, including reactive oxygen species (ROS)-mediated oxidative stress, cell membrane disruption, intracellular protein synthesis inhibition, and leakage of intracellular components. ROS mainly include superoxide (O2•–), hydroxyl radical (•OH), singlet oxygen (1O2), and hydrogen peroxide (H2O2), and generation of ROS by nano-materials is regarded as the main reason for nanomaterial-mediated antibacterial activity. Third, various nano-materials can act as antibiotic drug carriers to help effectively administer antibiotics to their target locations by reducing the probable adverse effects of antibiotics. Fourth, the retention power of nano-materials in the body is much more than antibiotics, which could be favorable for long-term therapeutic effects. Finally, nano-materials can be functionalized according to their target and purpose of use as they can be effective against bacterial cells without being toxic against mammalian cells [36]. Antibiotics encapsulated in NPs have a large capacity to be replaced with antibiotics in free form. There are numerous benefits in nano-encapsulation strategy such as very-small size, a big surface-zone-to-mass ratio, high loading capacity and high reactivity, protection of antibiotics against physical inactivation, improvement in antibiotics pharmacokinetics, facilitation of the antibiotic release in infection area and reducing the required dose of drug. The effect of aptamer-conjugated nanoparticles on the biofilm formation has been also evaluated. For example, single-walled carbon nanotubes (SWNTs) with significant antimicrobial activity were utilized as a nanomaterial to detect *Pseudomonas aeruginosa* as well as it could increase the toxic effect of antimicrobial agents on bacterial biofilms [37]. Nanotechnology has vast opportunity to command and modify molecular structures at nano-scale to attain specific target action. Nano-bullet targeting is advantageous over conventional systems as they enhance therapeutic capacity by preventing microbial resistance. Furthermore, nano-targeting often prevents frequent drug intake and reduces side effects. Therefore, nano-science enhances patient compliance through protecting natural microbiome. NPs have the capability to overcome drug resistance due to their multi-functionality, as bacteria cannot develop multiple gene mutations simultaneously [38].

Other approaches include use of genomics to find out new bacterial targets and optimization of newer approaches that target bacterial pathogens while exerting selection for reduced pathogenesis, if bacteria evolve resistance to therapeutic intervention [39]. An alternative approach could be to use gene-specific oligonucleotides, such as peptide nucleic acid (PNA) oligomers, that can specifically target any single pathogen and PNA as a nucleic acid mimic. This approach broadens the range of potential targets to any gene with a known sequence in any bacterium, and could significantly reduce the time required to discover new antimicrobials or their redesign, if resistance arises. Antisense oligonucleotides, used to inhibit the synthesis of proteins essential for bacteria to sustain life, may be helpful in the fight against bacterial infections. The peptide nucleic acid (PNA) molecule combines the properties of both peptides and nucleic acids. In the last two decades, many mRNA encoding essential genes in clinically pathogenic bacteria have been validated as possible targets for antisense PNA.

The PNA sequences can be used as a potential structure with antibacterial properties in gene expression. The first reported antibacterial PNA targeted the mRNA transcript of E. coli acpP gene that encodes the acyl carrier protein, a protein crucial in fatty acid biosynthesis. To improve the antimicrobial activity, PNA have also been used in combination with antibiotics. These included aminoglycosides, penicillins, polymyxins, rifamycins, sulfonamides, and trimethoprim. Other essential biological processes that have been disrupted by antisense PNA, in both gram-negative and gram-positive bacteria, are DNA transcription and replication. Respectively, the rpoD gene encoding RNA polymerase and gyrA encoding DNA gyrase were targeted by PNA in several pathogens including *S. pyogenes*, *S. aureus*, *S. Typhimurium* and *Shigella flexneri*. Nevertheless, the use of PNA as an antibiotic is not foreseen in the near future due to crucial limitations. The main drawback precluding the use of PNA as an antimicrobial is its lack of uptake by bacterial cells [40].

The acronym ESKAPE includes six nosocomial pathogens that exhibit multidrug resistance and virulence: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*. Persistent use of antibiotics has provoked the emergence of multidrug resistant (MDR) and extensively drug resistant (XDR) bacteria, which render even

the most effective drugs ineffective. Alternative therapies such as use of antibiotics in combination or with adjuvants, bacteriophages, antimicrobial peptides, nanoparticles, and photodynamic light therapy are widely reported. Some of the commonly described limitations of these therapies include stability and toxicity of the therapeutic agent, its targeted delivery at the site of infection, or immune response developed by the host against the therapeutic agent. Ongoing research has therefore led to further develop or modify these novel therapeutic agents or therapies so as to surmount the limitations as well as to overcome the barriers of bacterial resistance [41]. Consequently, the discovery and development of biofilm eradication agents as comprehensive and provide an overview of biofilm remediation strategies, focusing primarily on the most promising biofilm eradication agents and approaches [42]. Proteases secreted by equine mesenchymal stromal cells (MSC) are responsible for the degradation of proteins in MRSA biofilms. In addition to MRSA, was demonstrated that mature biofilms of P. aeruginosa, S. aureus, and S. epidermidis were reduced by MSC conditioned media (CM) as well. Furthermore, was demonstrated that equine MSC secrete cysteine proteases that destabilize MRSA biofilms, thereby increasing the efficacy of antibiotics that were previously tolerated by the biofilms [43]. However, the problem for Gram-negative bacteria is conceivably graver than Gram-positive pathogens due to these being more commonly multidrug-resistant (MDR). Thus, only six of these (ceftolozane-tazobactam, ceftaroline fosamil, ceftazidime-avibactam, meropenem-vaborbactam, delafloxacin and secnidazole) have been developed and found effective in the treatment of drug-resistant Gram-negative bacterial infections. The most widely studied hybrid compounds contain the fluoroquinolone class of antibiotic linked to another antibacterial agent. Since the last few years, many antibiotic hybrids have entered trials, but only a few have been reported to progress to clinical trials [44].

An alternative to killing bacteria or stopping their growth, is to search for drugs that disarm bacteria. This idea focuses on developing drugs that inhibit bacterial virulence rather than bacterial viability.

On the other hand, development of antivirulence therapies presents its own unique challenges. We can no longer use established screening systems that identify compounds that kill or inhibit growth of bacteria and minimal inhibitory concentration measures are obsolete in this scenario. Given that virulence mechanisms vary from one bacterium to another, antivirulence drugs are likely to have a narrow spectrum of activity. Their success in the clinic may well depend on development of real time diagnostics that identify the causative organism and enable therapy personalized to the infectious agent. However, aside from antibodies that inactivate specific bacterial toxins, none of these compounds with new mechanisms of actions has yet reached the clinic. So it remains to be seen whether all or some of these antivirulence approaches will live up to expectations [45]. Anti-virulence may offer a new wave of potential antibacterial therapeutics in the future, which drugs that will presumably have longer periods of clinical usefulness, compared to antibiotics. From the viewpoint of the development of virulence inhibitors, inhibition of quorum sensing (QS) is a promising route because various important features in bacterial physiology and virulence are mediated by QS-dependent gene expression (e.g., production of toxic shock syndrome toxin in *Staphylococcus aureus*, elastase in *P. aeruginosa*, protease in *V. cholerae*; activity of bacterial secretion systems (e.g., Salmonella species) and efflux pumps (e.g., *P. aeruginosa, Escherichia coli*), biofilm-production (e.g., *P. aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia*); induction of bacterial competence (e.g., Streptococcus pneumoniae), motility (e.g., *P. aeruginosa*), adhesion (e.g., *E. coli, Klebsiella pneumoniae*) and pigment-production (e.g., *Chromobacterium violaceum, Serratia marcescens, P. aeruginosa*) [46].

It is less well known that prophylactic vaccines also are highly effective and valuable tools to fight antibiotic resistance. Disease prevention by vaccination lowers antibiotic use and reduces resistance. Both *Haemophilus influenzae* type B (Hib) and pneumococcal conjugate vaccines are instructive examples and success stories having demonstrated their effectiveness in reducing antibiotic use and reducing resistance. Vaccines that are currently in clinical development differ from these earlier vaccines in that they are designed to address more comprehensively the complex pathophysiology of S. aureus infection by eliciting antibodies that target multiple virulence factors. Furthermore, the antibody responses elicited are functional and either kill the bacteria or neutralize the virulence factors [47]. While resistance is a predictable outcome of antibiotic use, resistance to vaccines

is an extremely rare event. One possibility is that vaccines typically restrict the ability of the pathogen to establish a foothold in the host, by conferring immunity before infection with the pathogen. Thus, vaccines reduce the chance that some bacteria mutate and become resistant, and also reducing the chance that these resistant genes are spread to other bacteria. The risk of a rare mutation occurring that allows the pathogen to resist the effect of vaccination is thus lower than it is with antibiotic treatment. In total, this overuse and/or misuse of antibiotics exerts selective pressure on bacteria overall, which facilitates the rise and transmission of resistance [48].

The advent of new bacterial genome engineering and synthetic biology (SB) tools is providing promising diagnostic and treatment plans to monitor and treat widespread recalcitrant bacterial infections. Key advances in genetic engineering approaches can successfully aid in targeting and editing pathogenic bacterial genomes for understanding and mitigating drug resistance mechanisms. The application of specific genome engineering and SB methods such as recombineering, clustered regularly interspaced short palindromic repeats (CRISPR), and bacterial cell-cell signaling mechanisms for pathogen targeting are currently essential. Apart from gene insertions, deletions or mutations for modification of the genome using engineering tools, one of the main goals of SB is to build and integrate gene circuits which process signals within a living cell for a desired output. The mobile group II introns are ribozymes that can insert into specific targets by the process of retrohoming. Using predictive algorithms, the intron RNA can be re-designed to form a 'targetron', such that a target DNA site of choice can be edited. This method has been adapted to a number of pathogenic strains for understanding mechanisms of virulence [49].

In any case, the war against antimicrobial resistance is not lost. We must continue the fight, which requires a better knowledge of the mechanisms of action of anti-infectious agents and concomitantly the mechanisms of resistance of infectious agents, a better and fairer use of antibiotics. We need "real" new molecules, i.e., with new chemical structures. Indeed, on 19 August 2019, the FDA approved the marketing of a brand-new antibiotic: Lefamulin, for the treatment of community-acquired bacterial-pneumonia (CABP). Lefamulin is the first antibiotic with a novel mechanism of action approved by the FDA in nearly 20 years. Lefamulin belongs to the family of pleuromutilins, which targets a different protein synthesis binding site than older antibiotics (i.e., it inhibits of protein synthesis by binding to the peptidyl transferase center of the 50S bacterial ribosome, thus preventing the binding of transfer RNA for peptide transfer). Lefamulin represents a great hope in the search for real new antibacterials. We must therefore continue to search for new antibacterial molecules and synthetic or natural molecules to feed the pipeline, because we will never stop needing new antibacterials [50]. The essential questions are now focused on the biology of the drug, with the PK/PD (pharmacokinetic (PK)/pharmacodynamic (PD) parameters) predictions of outcome in animal tests along with data from human volunteers and patients to act as surrogates for microbiological and clinical efficacy [51].

Conclusions. The development of new antibiotics is impeded by cut in financing by pharmaceutical companies the discovery of new antibiotic drugs. The reasons for these limitations in funding are both economic and scientific issues. Antibiotic resistance will continue to develop faster than appearance new antibiotics or validity the existent drugs available for treatment current infections. Bacteria elaborate new adaptive mechanisms frequently that can protect them against antibiotics. All of these need to investigate new antimicrobial strategies that become of vital importance. Manipulating the new approaches based on insights into metabolic and genetic properties of bacterial cell can enhance the possibility to withstand the proceeding antibiotic resistance. The goal in the future will be to explore the potential of some antimicrobial therapies and strategies to boost antibiotic applying. Advances genetic engineering approaches can be beneficial in targeting and editing pathogenic bacterial genomes for understanding and mitigating drug resistance mechanisms. Therefore, developing the new antibacterial, synthetic or natural molecules to feed the pipeline is a difficult ongoing question.

Conflict of interests. There is no conflict of interests.

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RELATION OF POSTMORTEM CHANGES DEVELOPMENT AND EXACT POSTMORTEM INTERVAL

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Abstract

Purpose: Precise postmortem interval evaluation is crucial in cases when violent types of death are suspected by a forensic medical examiner. There are different factors that could affect results of postmortem interval (PMI) evaluation by a forensic medical expert. The aim of this study was to investigate the relationship between the known postmortem time interval and the degree of particular postmortem changes development. **Materials and Methods:** A cross-sectional analysis of 116 forensic medical examinations of deceased persons (of them, 58 females and 58 males), in cases of non-violent death, was performed. The data about the time of death was obtained from police preliminary records provided to the examination - only the cases with known time of death were included in the study. Postmortem changes were evaluated by Total Body Score (TBS) [1] at equal time interval after death (48 hours ± 3 hours). Interconnection between postmortem changes degree and PMI was estimated using Spearman's rank correlation. Difference between sexes was evaluated using Mann-Whitney U test. **Results:** "Thickness of clothes" criterion reached the highest positive correlation coefficient, "ambient temperature" criterion had also a significant positive correlation. The rest of the studied criteria had very weak correlation with the development of postmortem changes. **Conclusions:** Several criteria had significant ($p < 0.05$), yet week, impact on the postmortem changes development. The other criteria were statistically insignificant.

Keywords: *forensic medicine, forensic medical examination, postmortem interval, thanatology.*

1. Introduction

Evaluation of the postmortem interval is one of the basic tasks of forensic medical expert. It plays a significant role in the work of police, due to a limited number of suspects who had an alibi when the crime occurred [2]. This evaluation is based on specific sequential changes that occur after death. The problem is that these postmortem changes vary due to many factors [3], including, but not limited to: ambient temperature, body weight, cloths, airflow [3, 4], and body length [5]. It could influence the estimation of PMI by a forensic medical practitioner, while performing examination [6], thus there is a need in defining the affective impact of these factors in order to further define new ways of making the PMI estimation more accurate as well as to elaborate methods which are already used by forensic medical experts.

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**Part of PhD thesis by Edgar Grygorian*

2. Purposes, subjects and methods:

2.1. Purpose. The aim of this study was to define the level of interconnection between development of postmortem body changes and the time passed after the death. We hypothesized that: 1) there is strong interconnection between them which varies depending on the thickness of clothes, weight of the corpse, body length, and environmental factors - ambient temperature, airflow; 2) development of postmortem body changes does not depend on sex and/or age of deceased person.

2.2. Subjects & Methods

Design

The study was based on 116 forensic medical examinations of deceased persons who died in a

result of pathologies (non-violent conditions) performed within the time period of 2017–2018 at Regional Bureau of Forensic Medical Examinations. Exclusion criteria were violent cases of death (accidents, suicides, homicides), cases where the forensic medical diagnosis was not specified, e.g. due to putrid decomposition, and cases of pathological conditions which were accompanied by massive hemorrhages. The study was approved by the ethics committee of Kharkiv National Medical University in October 2018. The practical part of the study was finished by July, 2019. During the study, the following parameters were gathered and analyzed: sex, age, length of body, fat development level, peculiarities of scene, type of clothes on the corpse. Evaluation of postmortem changes was made using TBS in 48±3, i.e. 46–51, hours after the death. In all cases studied, the time of death was obtained from police preliminary reports provided to examination.

Statistical Analysis

Mean (M) and standard deviations (SD) were determined. In order to check distribution normality, Shapiro-Wilk test was performed. As far as the tests showed non-normality of distributions, Spearman's rank correlation was used to calculate connections between grade of postmortem changes and studied criteria, as well as in calculation of differences in female' and male postmortem changes development. For each test the significance level had been set as $p < 0.05$. All calculations were conducted using Microsoft Office Pro Plus 2019, StatSoft STATISTICA Version 8 (Tulsa, OK).

3. Results

The results were approximated to three decimal places.

The greatest correlation was observed in "thickness of clothes" criterion (*Table 1, Figure 1)*. However, all the criteria studied had very weak or weak interconnections with the level of postmortem changes (i.e. less than 0.5), according to interpretation of Spearman rank correlation [7]. In particular, the mean of above mentioned "thickness of clothes" criterion was 0.320 – weak positive interconnection, "ambient temperature" criterion 0,249 (weak positive interconnection), "weight of corpse" criterion -0,055 (very weak negative interconnection), "body length" criterion -0,088 (very weak negative interconnection).

Analyzing and comparing outdoor and indoor scenes, it was found that, in cases with outdoor scenes, there was a weak positive correlation with postmortem changes development, while in cases with indoor scenes, there was a weak negative correlation. There was no information on the airflow level at the scene, so it was not possible to determine its impact on the postmortem changes` development.

Gender differences in females and males, in correlation with postmortem changes, were not significant – >0.1 ($p < 0.05$).

4. Discussion

Among the various methods of the postmortem interval evaluation, there is no specific one which could be precise enough to satisfy the needs of forensic medical examiners – to guarantee the adequate PMI evaluation in most of forensic medical examinations [1,3,8]. Scientists' examinations of pig carcasses as well as analysis of the investigations of human remains led to some controversial results. But even though some studies prove satisfactory results of currently used methods for some cases [3,9], there was one thing that was claimed in most studies – the methods should be modified in order to make the PMI evaluation more accurate [1,3,10,11]. Factors that should be evaluated are also varying in different studies, but there was no method that had gathered all factors affecting PMI and/or influence of these factors was not clearly measured [1, 3, 5–12].

Attempting to reach the maximum preciseness, the newly developed methods become more diverged from the currently used – e.g. bacteriologic tests [13], biochemical markers [14–20], immunohistochemistry [21, 22]. These methods, on the one hand, show positive results in PMI evaluation. On the other hand, most of such methods are time consuming and, like those using

Table 1

The correlation between various factors and the postmortem changes development (PMI = 48 hours ± 3 hours)

Age	Weight of the corpse	Body length	Thickness of clothes	Ambient temperature
<30	-0.081	0.017	0.231	0.215
30–39	-0.002	-0.251	0.363	0.150
40–49	0.077	0.062	0.229	0.314
50–59	-0.154	-0.147	0.420	0.312
>59	-0.114	-0.122	0.359	0.256

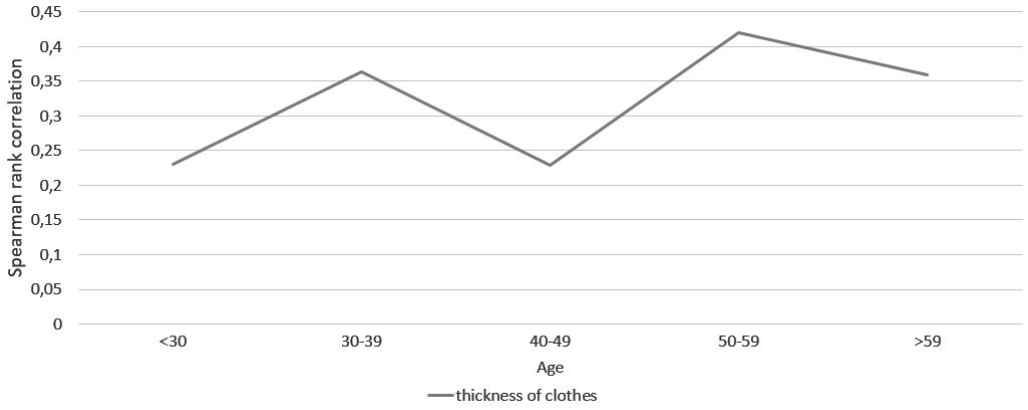

Figure 1. Spearman rank correlation of "thickness of clothes" criterion

immunohistochemistry, are expensive. Given the availability and quick assessment of PMI by currently used methods, such as TBS, it looks much more effective to supplement these methods first [23].

The limitation of the current study was the lack of complete information on the scene, which led to impossibility to measure all the additional factors affecting the postmortem changes of the body. Extensive data on scene peculiarities, such as airflow level, humidity, and also the more detailed information on the bedding of the corpse, sun exposure, could possibly let us to define their affection level on the PMI evaluation, with further comprehensive modifications to currently used methods.

Conclusion

During the study, it was found that there could be the impact of several factors on the development of postmortem changes. However, assuming the lack of information on the environment (airflow, humidity, sun exposure) in most studied cases, there is a need to evaluate the impact of these factors on the development of postmortem changes as well.

Declaration

The authors report no conflicts of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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EVALUATION OF THE STRUCTURE OF THE WALLS OF THE FRONTAL SINUS USING SPIRAL COMPUTED TOMOGRAPHY

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https://doi.org/10.35339/ic.7.2.76–80

Abstract

Introduction. The anatomical structure of the frontal sinus is of key importance for development of its inflammation as well as the complications with the spread to the adjacent organs and tissues (orbital phlegmon, brain abscesses, meningitis). The aim of our study was to compare the density and thickness of the bone tissue of the unchanged frontal sinus in various forms of chronic inflammation. **Materials and methods.** The study involved 121 patients with various forms of chronic frontal sinusitis: 56 with chronic hyperplastic (mucosal hyperplasia (up to 6 mm) and 33 patients with chronic purulent-polypous frontal sinusitis, manifested by a total and subtotal decrease in sinus pneumatization according to spiral computed tomography (SCT). 32 SCT samples were selected to form a comparison group without any abnormalities of the paranasal sinuses. **Results.** The maximum density typical for the lower wall of the frontal sinus under physiological conditions was found to be 107.96 ± 201.64HU, the minimum for the posterior wall in purulent-polypous frontal sinusitis was -103.74 ± 195.37HU.

The bone thickness both in the posterior region and in the region practically did not depend on the degree of the severity of pathological changes in it and was 1.0006 ± 0.538 mm, 0.91 ± 0.26 mm, 0.82 ± 0.169 mm under physiological conditions, with mucosal hyperplasia and with purulent-polypous frontal sinusitis in the posterior wall, respectively. In the region of the lower wall, 4.05 ± 2.04 mm, 2.32 ± 1.16 mm, and 4.002 ± 1.16 mm, respectively.

Conclusion. It can be assumed that the larger the change in PNSs, the lower the bone density. This in turn affects the prediction of possible complications during surgical treatment of chronic frontal sinusitis.

Key words: *frontal sinus, computed tomography, bone thickness, bone density.*

Introduction

The anatomical structure of the frontal sinus is of key importance for development of its inflammation and the complications with the spread to the adjacent organs and tissues (orbital phlegmon, brain abscesses, meningitis) [1]. It is the pathological processes that occur in the frontal sinus due to its topographic and anatomical relationships with nearby structures that most often lead to complications [2]. Their greatest occurrence occurs in chronic frontal sinusitis, because it is associated with bone changes in the sinus walls: bone demineralization, disappearance of trabeculae, cortical destruction, focal sclerosis [3]. These changes are manifested as a decrease in bone density, as shown by Dong et al. [4]. In addition to density, destructive processes often lead to a change in the thickness by the Global Osteitis Scale, in which the degree of destruction is associated with a decrease in the bone thickness [5]. These changes can correlate with severity of the disease, which must be taken into account when planning surgery for the maxillary sinuses and predicting possible complications [6].

2. Purposes, subjects and methods:

2.1. Purpose of our study was to compare the density and thickness of the bone tissue of the unchanged frontal sinus and in various forms of chronic inflammation.

2.2. Subjects & Methods

The study was performed within the framework of the planned complex research work of Kharkiv

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The study involved 121 patients with various forms of chronic frontal sinusitis: 56 with chronic hyperplastic (mucosal hyperplasia (up to 6 mm) and 33 patients with chronic purulent-polypous frontal sinusitis, manifested by a total and subtotal decrease in sinus pneumatization according to the findings of spiral computed tomography (SCT). The patients were selected with the same distribution by gender and age. The age of the subjects was 25 to 60 years. 32 SCT samples were selected to form a comparison group of people aged 25–60 without any abnormalities of the paranasal sinuses (see *Fig. 1*).

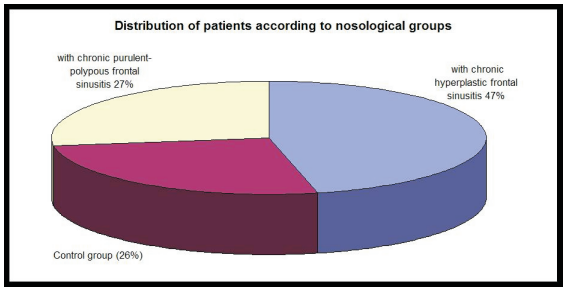


Fig. 1. Distribution of the patients according to nosological groups

This group of subjects underwent SCT examination due to conditions not related to ENT diseases. The article complies with the requirements of the Declaration of Helsinki. All patients were informed of their participation in the study and written informed consent to participate in the study was obtained. The study was approved by the Bioethics Committee of Kharkiv National Medical University.

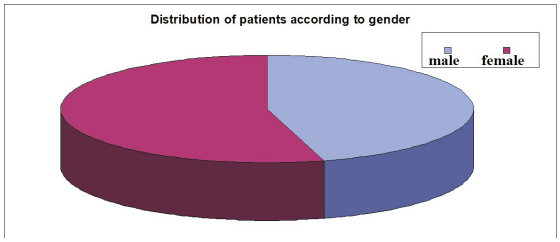


Fig. 2. Distribution of the patients according to gender

All patients underwent a generally accepted clinical examination according to the order of the Ministry of Health No. 181 dated 24.03.2009 "On the approval of medical care protocols in the otolaryngology". The Hounsfield scale [7] was used to evaluate bone density in SCT study, given that according to M. Hofer [8] modern devices are able to cover 4096 shades of gray scale, which represent different density levels in Hounsfield units (HU) (water density is taken as 0 HU, and air as 1000 HU). The calculation of the thickness was carried out in the thinnest section of the wall (see *Fig. 4*).

The obtained digital data were statistically processed using Student-Fisher method, the average value for each variation series (X), standard deviation, and the mean error (m) were determined. Statistical processing was carried out on a personal computer using Microsoft Office Excel 2010 software (USA). The results were considered statistically significant at $p < 0.05$.

Declaration

There is no conflict of interests. We certificate that we do not have any financial or personal relationships that might bias the content of this work.

3. Results & Discussion

The results of determining the thickness and density of bone tissue (HU) in SCT study using the Hounsfield scale are presented in *Tables 1–2*.

In the course of the study, the minimum thickness and density of the posterior of the frontal sinus were determined under physiological conditions and with varying degrees of severity of pathological changes in the sinus. The maximum density typical for the lower wall of the frontal sinus under physiological conditions and was found to be 107.96 ± 201.64HU, the minimum for the posterior wall in purulent-polypous frontal sinusitis was –103.74 ± 195.37HU.

The bone thickness both in the posterior region and in the region practically did not depend on the degree of the severity of pathological changes in it and was 1.0006 ± 0.538mm, 0.91 ± 0.26 mm, 0.82 ± 0.169 mm under physiological conditions, with mucosal hyperplasia and with purulent-polypous frontal sinusitis in the posterior wall, respectively. In the region of the lower wall, it was 4.05 ± 2.04 mm, 2.32 ± 1.16 mm, and 4.002 ± 1.16 mm, respectively.

In all cases the posterior wall was found to be much thinner than the lower, creating the conditions for the spread of purulent-inflammatory processes with the development of intracranial complications. The thickness of the lower wall (4.05 ± 2.04 mm) was shown to be 22.2% higher than the posterior (1.006 ± 0.538 mm) under physiological conditions (see *Fig. 3* A and B). Based on this, it can be assumed that chronic frontal sinusitis creates more favorable conditions

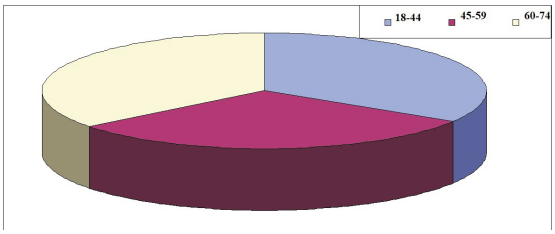


Fig. 3. Distribution of the patients according to the age

of maxillary sinuses, on the contrary, intraorbital complications may prevail over intracranial complications [10].

The study was performed using the findings of spiral computed tomography (SCT), which is a simple, informative, and generally accessible intravital method for determining bone density [11], used to identify the sizes and shape of the frontal sinus, distinguished by large individual and

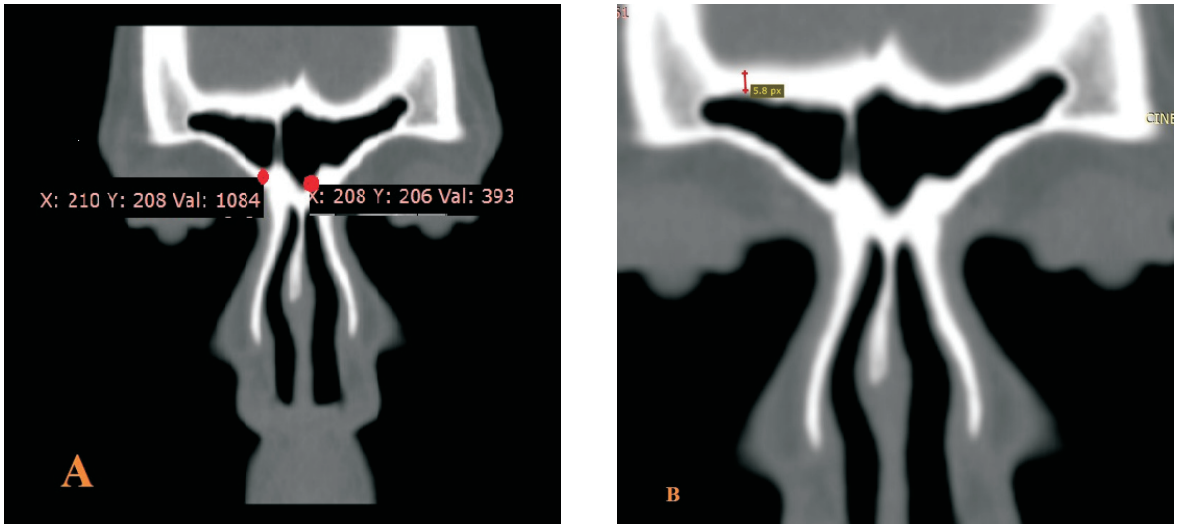


Fig. 4. An example of measuring bone density (A) and thickness. SCT, coronary reconstruction

Table 1

Density (HU) and thickness (mm) of the lower wall of the frontal sinus

Indicator	Without abnormalities		Parietal hyperplastic frontal sinusitis		Purulent polypous frontal sinusitis	
	Thickness	Density	Thickness	Density	Thickness	Density
M	4.05	107.96	4.002	2.68	2.32	-56.33
σ	2.04	201.64	2.07	160.88	1.16	78.51

Table 2

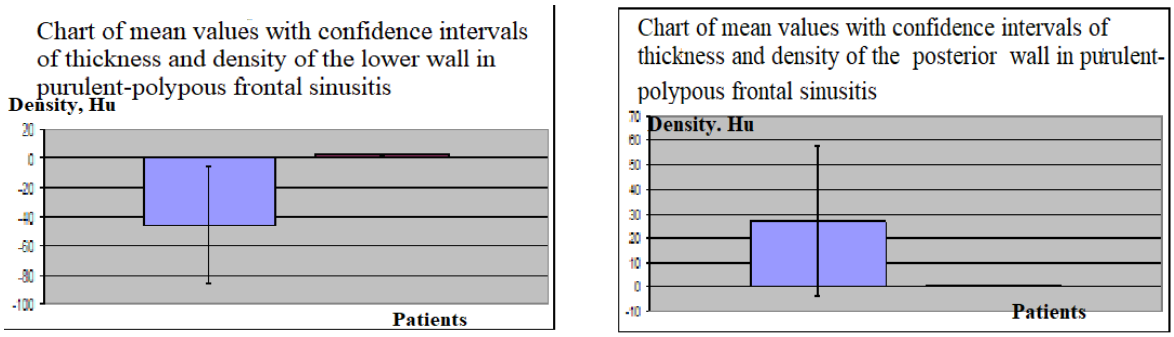
Density (HU) and thickness (mm) of the posterior wall of the frontal sinus

Indicator	Without abnormalities		Parietal hyperplastic frontal sinusitis		Purulent polypous frontal sinusitis	
	Thickness	Density	Thickness	Density	Thickness	Density
M	1.0006	27.42	0.82	-30. 29	0.91	-103.74
σ	0.538	168.76	0.169	202.4	0.26	195.37

for the spread of the inflammatory process intracranially than intraorbitally [9]. In addition, the likelihood of complications increased due to the lower density in the region of the posterior wall than the lower. Thus, the density of the posterior wall even under physiological conditions (27.42 ± 168.76 HU) was 25.4% lower than that of the lower one (107.96 ± 201.64 HU). In case

age-related variability [12]. SCT study helps to determine not only the main morphological aspects of the bone structure, but also to measure its density [13].

The study implied assessment of axial sections and coronary reconstructions. The thickness and density in the region of the lower (orbital) wall were calculated as the most significant in terms



A B

Fig. 5. Chart of mean values with confidence intervals of thickness and density of the lower (A) and posterior (B) walls in purulent-polypous frontal sinusitis

of the development of intraorbital complications [11], the posterior (cerebral) wall of the frontal sinus was studied, since it is of the greatest importance for endoscopic interventions [13]. There are data about changes of bone density depending of menopause [14].

As can be seen from *Tables 1* and *2*, bone thickness was a fairly constant indicator, less than the density changes under the influence of inflammatory changes in the sinus. Therefore, in assessing the degree of destructive changes in the sinus walls, we should rely more on density indicators [6, 15, 16]. In addition, both walls (both the lower and the posterior) were found to respond to the inflammatory process by decreasing the density, and it was most pronounced with the maximum degree of severity of the inflammatory process in the sinus, which was associated with the development of purulent-polypous frontal sinusitis. Thus, bone density depended on the severity of changes in the PNSs, since in the presence of a cyst it was maximal and slightly different from that normal. In purulent polypous process, it was minimal.

Conclusions

1. Our findings demonstrate that SCT can determine the minimum density and thickness of the lower and posterior walls of the frontal sinus under physiological conditions and with varying degrees of severity of inflammatory changes in it.

2. The posterior wall is the thinnest among all PNSs in all the studied groups. In addition, it has minimum density, which creates the conditions for the spread of purulent-inflammatory process in the skull.

3. It can be assumed that the larger the changes in PNSs, the lower the bone density. This, in turn, affects the prediction of possible complications during surgical treatment of chronic frontal sinusitis.

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NEGATIVE EXPERIENCE IN BLOCKING INTRAMEDULLARY OSTEOSYNTHESIS
(REVIEW)

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Abstract
Treatment of diaphyseal bone fractures is a complicated, controversial and ambiguous task. Blocking intramedullary osteosynthesis, which became the standard of treatment, is also not perfect and accompanied by a number of complications. The reasons for their occurrence are connected both with defects in the organization of treatment of patients, and with tactical mistakes, the definition, generalization and prevention of which became the purpose of our study.
It was found that typical tactical mistakes were use of the method against the indications, non-compliance with the technology of blocking intramedullary osteosynthesis and the use of unsubstantiated and inadequate "proprietary methods", making their own "modifications" during the surgery and changing the course of operative intervention, excessive drilling of the bone marrow canal, significant intraoperative traumatism of bone fragments and surrounding soft tissues, incorrect type of lock or timely unfulfilled dynamization, lack of consistency and restorative and rehabilitative treatment.
But the most negative effect on the anatomical recovery of the bone and functional recovery of the extremity was the bone marrow canal drilling, performed without the corresponding indications and technical disadvantages. It is obvious that the violation of the endostosis of the endostal negative effect on the reparative capabilities of bone tissue, inhibits the process of bone grafting and delay recovery.
Therefore, there is no doubt that the further study of the effect of bone marrow duct penetration in the course of reparative osteogenesis is relevant and appropriate.
Key words: *blocking intramedullary osteosynthesis, tactical mistakes, fracture, long bones, drilling.*

Orthopedic and trauma pathology is ranked second internationally after cardiovascular diseases and first among the causes of incapacity and primary disability [1]. Extremity injury is the most common injury, most of them (from 50,4 to 72,1% of all injuries of the musculoskeletal system) are the long tubular bone injuries [2], while lower extremity fractures occur twice as often as the upper limb fractures [3].

The majority of the long-bone fractures are diaphyseal fractures [4]. Among the population of Ukraine, diaphyseal fractures make 48.5% of all long-bone fractures [5]. As for the location of long-bone shaft fractures, tibia fractures (40–56%) are on the first place, followed by femur fractures (25–34%); forearm and shoulder fractures 14–20% and 11–17%, respectively [6, 7].

Despite the large number of studies on this topic, management of diaphyseal bone fractures is a complicated, controversial and ambiguous task. Since several different techniques can be used to treat one fracture, it usually results in mistakes which delay the recovery of the patient and affect negatively on the final outcome. Disruption of fracture union process has a serious impact on the quality of life of the patient, the term of disability, increases the risk of development of local and/or systemic complications, and is a

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burden for the health system and the family of the patient [8, 9]. The researchers note the lack of explicit dynamics in reducing the frequency of complications in treatment of extremity fractures [10].

The outcome of long-bone fractures at the present depend on the set of objective and subjective factors: the age of the patient, the type and degree of concomitant pathology, localization and type of fracture, the time elapsed from the moment of trauma to the operation, the method of fixation of fractured bone fragments and many others [11].

Despite the advantages of the operative method, treatment of long-bone fractures is often accompanied by a number of complications. Causes of complications are associated with defects in the management of patients (improper treatment tactics, wrong choice of the method of osteosynthesis, disturbances of recovery regimen in the postoperative period, patients non-compliance of the term of limb loading, etc.), as well as with technical errors associated with the actual performance of the operation (traumatic surgery, instability of osteosynthesis, wrong choice of metal constructions, insufficient hemostasis, etc.) [12].

M.O. Korzh et al. [13] concluded that the most common medical errors are the underestimation of the severity of the damage; inconsistency of the applied method of treating the traumatic injury and the patient's condition; incomplete repositioning of fractured bone fragments; use of implants made of low-quality material; violation of osteosynthesis technique, resulting in lack of stability in "bone-bone" and "bone-implant" systems; lack of consistency throughout all phases of treatment and an unreasonable change in the treatment method; as well as, inadequate medical rehabilitation.

In general, complications after osteosynthesis of long bones extremity are divided into local (in the location of the surgery) and general; as well as the infectious and noninfectious [14]. The following complications in the process of treatment by osteosynthesis are described in the literature: operative wound abscess, delayed consolidation, malunion, pseudoarthrosis, soft tissue irritation in the location of fixator or plate, fracture of the metal constructions, the false joint formation, debris syndrome, compartment syndrome, osteomyelitis [15, 16].

Other complications include intraoperative (secondary) fractures, delayed union, reduced contact strength of screw carvings with bone, secondary displacement of bone fragments, migration and fracture of structures [17, 18].

Instability of the bone damage zone, especially in the presence of a metal implant, can have the most severe destructive effects, namely the formation of a large periosteal callus, which calls fracture consolidation into question; widespread resorption of bone tissue, resulting in the formation of a false joint. Conversely, in the conditions of stable osteosynthesis and the preservation of the property of a fixing metal anchor and bone fragments, an osteoinductive effect of the implant was detected.

Literature data analysis shows that the issue of complications after treatment of long-bone fractures has captured the attention of many researchers. Complications undoubtedly influence both the outcome and the patient's life quality. The prognosis of the development of complications after long-bone surgery is possible, but in most cases, it is carried out on the basis of subjective experience of traumatologists. In general, when analyzing data from domestic and foreign literature it is clear that the incidence of complications in the treatment of long-bones fractures is still rather high. To prevent adverse treatment outcomes is possible on the basis of predicting and preventing system. However, in the literature such information so far can be found in separate publications only [19].

At the present stage, the gold standard for the treatment of diaphyseal bone fractures is de facto blocking intramedullary osteosynthesis. The main advantage is its low traumaticity, since the nail in the marrow canal is introduced far from the seat of fracture, which makes it possible to preserve the source of periosteal blood supply, which is important in the process of subsequent fracture consolidation [20, 21]. The promising outcome, the creation of unified tools and sparing techniques contributed to the rapid spread of closed blocking intramedullary osteosynthesis in the number of developed countries [22].

Significant advantages of blocking intramedullary osteosynthesis, such as low invasiveness, lack of intraoperative blood loss, significant stiffness of fixation, high quality of life, the absence of need for regular physical therapy for the development of joint movements practically minimize the risk of complications in the treatment process, make this technique optimal for the treatment of diaphyseal long-bone fractures [23].

The philosophy of operative therapy with the use of closed intramedullary osteosynthesis with blocking provides the possibility of stable fixation of bone fragments in an anatomically correct position without intervention in the place of fracture; the implementation of early dosed

physical exertion on the operated extremity, the implementation of passive and active movements in adjacent joints without any additional external immobilization [24, 25]. The advantages of intramedullary osteosynthesis include the lack of discomfort, the possibility of self-sustaining self-care and an independent move, and a reduction in the length of stay in a medical facility [26].

The undeniable advantages of closed blocking intramedullary osteosynthesis are also relative simplicity of surgical intervention and primary stability of fractured bone fragments [27, 28]. All these factors contribute to the consolidation of fractured bone fragments, rapid household and social adaptation of the patient with the possibility of a quick return to work [22]. According to [7, 29], currently blocking intramedullary osteosynthesis with diaphyseal fractures has the right to be considered as the classical treatment method of this category of damage. It should be noted that this treatment method allowed 95% of patients to receive positive outcome [30].

However, it is known that, like any other treatment method, blocking intramedullary osteosynthesis is not devoid of deficiencies and is accompanied by a number of complications [31]. Unsatisfactory results can be explained as separate shortcomings of operational techniques – inadequate selection of clamps, incorrect technique of osteosynthesis, insufficient repositioning of fragments, etc., especially, and tactical mistakes, such as, the inconsistency of the fracture with this method of osteosynthesis, the choice of an incorrect method of blocking, the presence of related injuries, which may influence the choice of the method and the term of osteosynthesis, marrow canal drilling without indications or, conversely, a refusal to drill in the shown for these cases.

Further study of the effect of bone marrow drilling in the course of reparative osteogenesis is relevant and appropriate. Knowledge of typical tactical mistakes and adherence to the method of blocking intramedullary osteosynthesis may reduce the number of poor anatomical and functional results and avoid complications.

Conflict of interests

The authors state that there is no conflict of interest in the preparation of this article.

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ENDOSCOPIC TREATMENT OF ESOPHAGEAL ANASTOMOTIC STENOSES AND LEAKAGES

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Abstract
Introduction. Endoscopic stenting of esophageal anastomosis due to anastomotic stenosis or leakage is increasingly being used as one of the most effective, minimally invasive and safe methods of treatment. **Materials and methods.** This research is based on the experience of treatment of 49 patients with gastric and esophageal cancer who previously were operated at the clinic and had complications such as esophageal anastomotic leakage and stenosis. Anastomotic leakage was observed in 21 cases: 9 patients were with gastroesophageal anastomosis, 12 patients were with esophagointestinal anastomosis. Stenosiss of esophageal anastomosis were observed in 38 cases: 20 patients were with gastroesophageal anastomosis, 18 patients were with esophagointestinal anastomosis. **Results.** All patients were undergone endoscopic stenting of esophageal anastomosis. The results of using this method of treatment were estimated. **Conclusions.** Stenting of the esophageal anastomosis by coated self-expanding stents is a method of choice in the treatment of patients with esophageal anastomotic leakage and stenosis.
Key words: *anastomotic leakage, anastomotic stenosis, esophageal resection, gastrectomy, stenting of esophageal anastomosis, subtotal proximal gastrectomy.*

1. Introduction

Gastroesophageal surgery currently remains one of the most technically challenging areas in the digestive tract surgery. It is associated with high rates of postoperative mortality, which ranges after gastrectomy and esophageal resection from 3.3 to 26.1% [1–3]. The gastroesophageal anastomotic leakage is observed in 2.2–5.91%, esophagointestinal anastomotic leakage – in 6.3–32.0% of patients [3–8]. Anastomotic stenoses are formed after gastrectomy, resections of the esophagus in 9–30% of patients [7–13]. Surgical treatment of esophageal anastomotic stenoses has 25% mortality [10]. In connection with this, in recent years, the priority has been given to the search of more effective minimally invasive methods of the treatment of esophageal anastomotic stenosis [9, 10, 14]. This has contributed to the creation of certain devices, the development and application of endoscopic technologies, which are currently leading in the treatment of the esophageal anastomotic stenoses [9–11, 15–16]. The used methods of recanalization of the esophageal lumen in the region of cicatricial anastomotic stenosis are the following laser, electro-, argon-plasma coagulation, balloon dilatation and bougienage [1, 2]. However, despite the fact that in most cases it is possible to expand the lumen in the constriction zone, it is necessary to perform repeated manipulations due to the impossibility of achieving a positive persistent effect in one session [10–11]. Endoscopic stenting of esophageal anastomosis is increasingly being used as one of the most effective, minimally invasive and safe methods of treatment [15–17]. Moreover, the use of this technique in case of esophageal anastomotic leakage often contributes

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to saving of the patient's life [3–6, 14, 17]. For this purpose, self-expanding nitinol stents are used [15–16]. The advantage of these stents is also the presence of anti-migration mechanism in some models. Also, the reflux of gastric or intestinal contents into the esophagus is eliminated by the integrated anti-reflux valve [6, 15–17]. In addition, it is believed that the stenting of nitinol self-expanding stents is a sufficiently effective and safe procedure in treatment of post-operative complications in the esophageal anastomosis region such as anastomotic stenosis and anastomotic leakage [4–6, 14, 17, 18].

2. Purposes, subjects and methods

2.1. The purpose of the researche was to analyze the results of the application of stenting of the esophageal anastomoses in patients with esophageal anastomotic stenosiss and leakages.

2.2. Subjects and Methods

For the period from 2006 to 2019 we accumulated experience in the use of stents in treatment of post-operative complications in the esophageal anastomosis region such as anastomotic stenosis and anastomotic leakage. Stenting of anastomosis was performed for treatment of 38 patients with esophageal anastomotic stenosis and 21 patients with esophageal anastomotic leakage. 18 patients with cicatricial esophageal anastomotic stenosis previously underwent gastrectomy due to stomach cancer, 4 patients – subtotal proximal gastrectomy due to cancer of the cardiac part of the stomach, 10 patients – esophageal resection with Lewis esophagogastroplasty due to esophageal cancer, 6 patients – esophageal resection with Harlock esophagogastroplasty due to esophageal cancer. 11 patients with esophageal anastomotic leakage previously underwent gastrectomy due to stomach cancer, 4 patients – subtotal proximal gastrectomy due to cancer of the cardiac part of the stomach, 5 patients – esophageal resection with Lewis esophagogastroplasty due to esophageal cancer, 1 patient – combined gastrectomy with gastroplasty by the ileocecal segment.

The stents by M.I.Tech Co., Korea: – 29 and Boston Scientific Corp., USA: – 30 were used for the stenting of the esophagus.

At the initial stages, the stenting were performed by the X-ray control with angiographic mode. Patients took in a water soluble radiopaque solution for visualization of anastomotic leakage zone or to determine the beginning of cicatricial stenosis of anastomosis and its length. Radiopaque dermal labels were placed on the skin. Also endoscopic examination was performed. Then string-guide were introduced distally to the anastomosis. The endoscope was removed. After the conduction of the delivery device along the string by the X-ray control to the required level, the stent was released. After the final disclosure of the stent, X-ray and endoscopic controls were also performed. With the development of the technology the stenting procedure began to be performed only under visual endoscopic control followed by X-ray control after stent placement.

Conflict of interests

The authors of the article declare no conflict of interest.

3. Results

Successful stenting were performed in 100% of cases. Clinical effect was achieved in all patients. Immediately after stenting, patients could take the liquid with the subsequent returning to the recommended diet.

Then local complications of patients with esophageal anastomotic leakage were treated: local peritonitis as a result of anastomotic leakage was treated by antibacterial therapy and sanation of the abdominal cavity through the inserted drainages. Treatment of pleural empyema in the zone of leak due to the gastroesophageal anastomotic leakage after esophageal resection in addition to antibiotic therapy included irrigation and drainage of the pleural cavity with the transition to the puncture method under ultrasound monitoring after gradual delimitation of the inflammatory process.

4. Discussion

All patients with esophageal anastomotic leakage were discharged from the hospital without signs of anastomotic leakage in a satisfactory state.

All patients with esophageal anastomotic stenoses after stenting had good functional results. Immediately there was a significant reduction of dysphagia. In 3–5 days after stenting dysphagia disappeared completely.

After stenting 4 cases of stent migration were observed: in one case – proximal migration, 3 cases – distal migration. Reposition of the stents were performed with a positive result.

Such treatment results can be comparable with scientific reports of another researchers [6, 9, 10, 17, 18].

Assessment of patients' life quality and their treatment efficiency consisted in the evaluation of gastroenterological patients' treatment efficiency at the different time periods of treatment by objective and subjective parameters and their integral estimation by the method of evaluating the effectiveness of treatment of patients with gastrointestinal diseases [19, 20].

Assessment before stenting evidenced the lower life quality in all cases. At 10th day after stenting and later on the 20th and 30th day, all objective and subjective life quality parameters confirmed a higher treatment efficiency and better life quality in 34 (91,84%) patients. Lower life quality parameters in 4 (8,16%) cases were caused by post stenting complications [19].

Long-term complications in patients after stenting were absent. This is an evidence of substantially better long-term results of such treatment tactics.

Within the subsequent period after stenting in 1, 3, 6, 9, 12 months all life quality parameters reflected a better life of the patients [19].

Conclusions

1. Stenting of the esophageal anastomosis by coated self-expanding stents is a method of choice in the treatment of patients with esophageal anastomotic leakage and allows to avoid traumatic operations, especially for the weakened patients. Also it allows to save lives of the patients with these severe complications.

2. Stenting is a very effective miniinvasive method of treatment of the cicatricial esophageal anastomotic stenoses. Especially it is useful when other methods of treatment were unsuccessful (bougienage, balloon dilatation), that allows to restore the lumen of the gastrointestinal tract and improve the quality of life of the patients. Also it is an alternative to traumatic operations of correction of the esophageal anastomotic stenoses.

3. Endoscopic stenting of the esophageal anastomosis by coated self-expanding stents in patients with esophageal anastomotic stenosis or leakage contributes to the improvement of treatment results and life quality of operated patients.

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THE FEATURES OF CENTRAL NERVOUS SYSTEM DAMAGE IN CHILDREN WITH CONGENITAL CYTOMEGALOVIRUS INFECTION

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Abstract
The paper discusses the features of central nervous system lesions in children with congenital cytomegalovirus infection at different stages of gestation. In the course of the work, an algorithm for diagnosing congenital cytomegalovirus infection was developed. To demonstrate the complexity of diagnosis and treatment in a child of the first year of life, a clinical observation is given. The positive dynamics in the treatment of children with the use of human anticytomegalovirus immunoglobulin with a high content of IgG is discussed.
Key words: *damage, central nervous system, congenital, young children, anticytomegalovirus.*

Introduction

For several decades, herpes virus infection has remained an urgent multidisciplinary problem in pediatrics, infectology, obstetrics and gynecology. According to the World Health Organization (2018), a pandemic of herpes virus infections is observed: 90% of adults and children on the planet has been infected with herpes viruses, and 50% of them have a manifest, recurrent course [1].

Human cytomegalovirus (CMV) is a β-herpesvirus that can cause a primary infection. For the first time in the laboratory, the virus was cultivated by M. Smith (1955). Infection of infants with CMV occurs with breast milk in 57% [Kulikov VI, 2017], through saliva and blood transfusions, during transplantation of internal organs and hematopoietic stem cells, through the affected genital tract of the mother in 63% [Karazhas NV , 2015]. According to the American Public Health Center, of 4 million births per year in the United States, about 1% of children with congenital CMV infection are born; 8700 children annually have early and late complications of CMV infection [Frize K. 2013]. According to Walker S. P. (2013), 3.7–20% of pregnant women in the United States were allocated CMV in the material taken from the cervical canal. The incidence peak encompasses two time intervals: up to three years – 39% of patients; 16–25 years – 41%. [2] In the study of CMV infection in pregnant women, primary CMV infection was found in 32.3% of women with the risk of termination of pregnancy, in 18.1% – spontaneous abortion, in 39.5% – premature birth, in 54.4% – with signs of polyhydramnios, 35,2% – with a dead pregnancy [Revello M. G 2015]. After infection, CMV is often transformed into a latent form with periodic reactivation in the host organism [3]. In immunocompetent people, the infection is asymptomatic, and in some patients has clinical manifestations of infectious mononucleosis, splenomegaly is diagnosed. Despite the fact that CMV is the main infectious cause of sensorineural hearing loss and abnormalities of the development of the central nervous system (CNS), congenital CMV infection is poorly diagnosed and is detected only in 0.2–2.0% of pregnant women [4–6] .

2. Purpose, subjects and methods:

2.1. The purpose of the study was to analyze modern medical literature on CNS disorders in children with congenital CMV infection.

2.2. Subjects & Methods

The basis of the study is the study of open scientometric and abstract databases PubMed,

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HINARI, Google Scholar using bibliographic and analytical-syntactic methods.

Conflict of interests.

There is no conflict of interests

3. Results & Discussion

Informal International Congenital Cytomegalovirus Recommendations Group (ICCRG) congenital CMV infection group identified major risk factors for disease (2015), including: lack of maternal or newborn screening programs, low level of routine testing of infants who are at risk and parents about infections during pregnancy, limited efficacy and high toxicity of modern therapies during pregnancy, the absence of licensed vaccines [7–9].

CMV infection is a viral disease characterized by polymorphic clinical symptoms resulting from the formation in the salivary glands, visceral organs and the CNS of specific cytomegal cells.

The International Classification of Diseases (ICD-10), distinguished the following CMV infections:

P35.1 Congenital CMV infection

B25 Cytomegalovirus disease

B25.0 Cytomegalovirus pneumonitis

B25.1 Cytomegalovirus hepatitis

B25.2 Cytomegalovirus pancreatitis

B25.8 Other cytomegalovirus diseases

B25.9 Cytomegalovirus disease, unspecified

In clinical practice, the following classification of CMV infection is used:

1) by nature of infection: congenital, acquired;

2) by the course: acute (up to 3 months), subacute (3-6 months), prolonged (6–12 months), chronic (more than 12 months);

3) in the form: localized, distributed, mixed, generalized;

4) by stages of the disease: active, manifestations, relapse with activation period, active stage, stage of convalescence, stage of compensation.

CMV can be transplanted translucently when a woman develops a primary CMV infection during pregnancy or as a result of latent reactivation of the virus acquired prior to pregnancy. [10]. The risk of transmission of fetal infection is higher among pregnant women with primary infection compared to those who were IgG-positive for pregnancy. Vertical transmission of CMV fetal infection is more common as a result of maternal infection in the third trimester of pregnancy, but the incidence of CNS complications is lower among children infected in the third trimester than infants born to women with primary CMV infection in the first trimester [11 , 12].

Depending on the gestation period, CMV infection is manifested by different clinical symptoms. When infected in the period from 16 to 75 days, embryopathies develop, because CMV exhibits high neurotropism, almost 60% of cases of manifestations of embryopathy are organic CNS lesions in the form of: hydrocephalus – 24%; microcephaly – 15%, ventriculomegaly – 11%, hypoplasia of brain structures – 6%, delay of myelination – 4%, and others [13]. The disorders of the cardiovascular system, in the form of defects in the development of the valvular apparatus of the heart, defects of the atrial and interventricular membranes, are equally important. Infection within the period from 76 to 180 days results in development of fetopathies, which are manifested by fibro-sclerotic deformities of the organs – 15%, miscarriage – 5%, hepatitis – 23%, encephalitis – 11%, paraventricular cysts – 4%, calcification of the substance of the brain %, chorioretinitis – 7%, neurosensory hearing loss – 27%, pneumonia – 5% [14].

The nature and timing of the manifestation of congenital CMV infection depends on the premorbid condition of the newborn, namely, the burden of obstetric history, immaturity, the presence of hereditary diseases, concomitant pathology, in weak, prematurely born children, manifestation of infection can be observed 4–5.

The first clinical signs of congenital CMV in newborns are manifestations of CNS lesions in the form of: suppression of unconditional swallowing reflexes, suction – 21%, focal symptoms and manifestation of strabismus – 16.3%, nystagmus – 27%, asymmetry of facial muscles – 6%, muscle tone in the form of: hypertonicity – 13.1%, hypotension – 29.8%, or mixed form – 10.1%, in the presence of hydrocephalus, there are signs of increased intracranial pressure, namely: a significant increase in the circumference of the baby's head in a short time – 13,2%, blasting of the thigh – 22,9%, with "fountain" regurgitation – 24.8%, convulsive syndrome – 7%.

According to Grosjean J. (2014), children with CNS lesions showed signs of encephalitis (31.2% of cases), which manifested from the second week of life and had a wavy course with early in 14.1% and late in – 23.4% exacerbations. Children with encephalitis had manifestations of intoxication, impaired consciousness in the form of inhibition (27.4%), as well as resistance (19.6%). Focal symptoms in the form of horizontal nystagmus – 35.4%, strabismus – 8.2%, vomiting – 12.3%, convulsive syndrome – 17% appear approximately one week after the onset of the

disease, requiring additional vigilance for the diagnosis of encephalitis in children with congenital CMV infection.

In the majority of children (up to 85%), congenital CMV infection occurs in subclinical form. In 35% of these children manifestation of congenital infection with CNS lesions is observed for 3–7 years of life and manifests neurosensory hearing loss – 41%, visual impairment in the form of partial – 19,1%, or complete atrophy of the optic nerve – 24%, convulsions –23,5%, pseudobulbar syndrome – 6,7%, and episyndrome formation – 11,7%. There are also motor disorders by type of pyramidal insufficiency – 60.7%, pediatric cerebral palsy – 23.1% of children, delay of speech and psychomotor development – 80.1%, disorders of behavior – 5%.

According to foreign literature, up to 12 % of infants with clinical manifestations of CMV infections die, and most newborns who are infected early in pregnancy die within the first 10 days of life, and even in such a case it is difficult to identify the cause of death and verify the cause. According to the literature, 73.8% newborns with the generalized form of congenital CMV and 9.5% with cerebral one tend to die [15]. Thus, the number of deaths in the first month of life is 37.1%, at the age of 1–3 months – 18.3%, 4–6 months – 4.8%, 7–12 months – 1.6%. According to the pathoanatomical study, in 51.6% of the dead, there were signs of pre-natal infection in the form of: low body weight, encephalitis, meningitis, calcifications of the substance of the brain, hepatitis, many fetodysplasias, such as aortic hypoplasia, kidney, dysplasia valves, premature closure of fetal communications [16].

According to Prince HE (2014), the pathomorphological study of the placenta of patients with congenital CMV infection revealed subchoral intervals – 21.1%, purulent widespread chorioamnionitis – 10.5%, accelerated maturation of chorionic villi – 14.7% blood flow – 6.1%, which led to fetal hypoxia and delayed prenatal development.

It is recommended to do the test on seroconversion, IgM antibody studies and IgG avidity index for CMV for the baby from the pregnancy with a flu-like symptoms (fever, severe fatigue, headache) that do not related to another established infection, and also for the fetus with a suspected CMV infection by the results of ultrasound examination [17].

To demonstrate the complexity of diagnosis and treatment in a child of the first year of life, we present the following clinical observation.

A 5-month- old patient was brought to the regional children's clinical hospital because of delayed psychomotor development. The baby was born from the first pregnancy (against smoking of the pregnant woman and infection with herpes virus infection with episodes of fever in the third trimester of pregnancy, delayed fetal fetal development), at 37 weeks, with a body weight of 2750 g, body length 50 cm.

Previously at the age of 2 months she was hospitalized because of fever to 39.1, mucous discharge from the nose, dry cough, delayed psychomotor development in the form of suppression of reflexes, muscle , inability to hold head, strabismus. The treatment resulted in improvement of the child's condition, the patient was discharged from the hospital.

At the age of 4 months, she was re-hospitalized at a regional children's clinical hospital because of fever, catarrhal manifestations from the upper respiratory tract. General condition of the child was severe due to fine intoxication. Psychomotor development did not match the age of the baby, there was jaundice of the skin, hepatosplenomegaly. Clinical blood test showed leukocytosis with a shift of the leukocyte formula to the left, accelerated ESR, monocytosis. Biochemical analysis of blood demonstrated five-fold increase in the liver tests (ALT, AST). Three-fold increase of anti-CMV IgM and IgG was revealed by enzyme immunoassay.

Congenital CMV infection with atypical hepatitis and developmental delay was diagnosed.

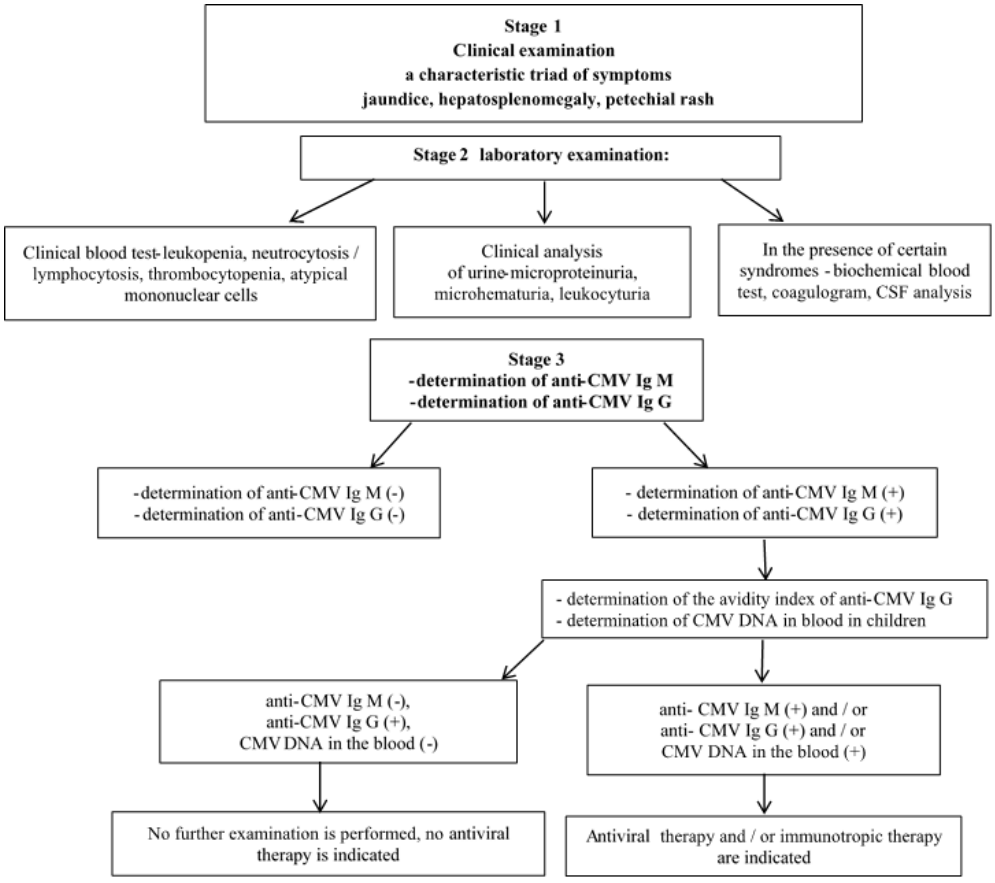
After the treatment, positive dynamics was noted and the patient was discharged from the hospital.

At the age of 5 months, she was admitted to the hospital due to delayed psychomotor development. Upon admission, the condition of the child was of moderate severity, the consciousness is clear. There were signs of delayed psychomotor development, namely, poorly expressed orientation in space, voice response was reduced, unconditioned reflexes were expressed weakly, there was muscular dystonia, inability to roll over, poor head retention, decreased reaction of sensorimotor behavior, squinting hepatosplenomegaly.

Clinical blood test showed Hb – 92 g/l, RBC – 3,6 × 1012 / l, blood quotient – 0,8, WBC – 9,5 × 109 / l, eosinophils – 1%, rod-core neutrophils – 1%, segmented neutrophils – 48%, lymphocytes – 40%, monocytes – 10 %, the erythrocyte sedimentation rate was 15 mm / h. clinical urinalysis demonstrated the signs of leukocyturia (leukocytes in the entire field of vision). Biochemical blood test showed total protein – 62 g/l, alanine

aminotransferase – 68 units/l, aspartate aminotransferase – 95 units/l, cholesterol – 3.8 mmol/l, the level of nitrogenous slag: creatinine – 0.052 mmol/l, urea – 2 , 9 mmol/l. Enzyme-linked immunosorbent assay revealed anti-CMV IgM and IgG. PCR revealed the viral DNA in the blood.

To diagnose CMV infection we developed and used a diagnostic algorithm which is presented in *Figure*.



Diagnostic algorithm of CMV infection

In view of the foregoing, a clinical diagnosis the following diagnosis was made: "Congenital CMV infection. Atypical hepatitis. Anemia of mild severity. Uncomplicated pyelonephritis. Delay in psychomotor development".

Treatment of CMV infection consisted of etiotropic and symptomatic therapy. According to modern treatment protocols, etiotropic therapy involves administration of antiviral drugs: Ganciclovir (cymeven) 5–7.5 mg/kg / day in 2 doses with an interval of 12 hours, the course of treatment 14–21 days; recombinant Interferon alfa-2 (Viferon) rectal suppositories of 150000 IU, or Foscarnet (a synthetic antiviral drug that suppresses DNA polymerase) is used as a second-line drug in the event of resistance to ganciclovir or due to side effects (level of evidence B).

Specific anticytomegalovirus immunoglobulins are prescribed together with antiviral drugs. Detoxification therapy is performed depending on the degree of severity, if a mild and moderate degree prescribe copious drinking in the form of fruit, vegetable juices, then in severe condition requires the use of infusion therapy. Regarding the effectiveness of the treatment, there is a regression of clinical symptoms, according to laboratory studies - the absence of virus DNA in the blood, the absence of anti-CMV Ig M and anti-CMV IgG with low avidity, the presence of anti – CMV IgG with high avidity [18].

Cytotect (Biotest Pharma GmbH, Germany), a human anticytomegalovirus immunoglobulin with high Ig G content (evidence level B) was administered. These antibodies are complementary to glycoproteins that are on the membrane of the virus. Neutralization of these glycoproteins makes it possible to prevent the damage to healthy cells and to reduce further virus replication.

The cytotect was administered as IV drops 2 ml/kg every 48 hours. The course of treatment of the child consisted of 5 injections. Dynamic observation after the first administration demonstrated a noticeable regression of neurological

symptoms, normalization of the orientation in space, increased muscle tone, appearance of the ability to turn and hold the head. The patient was discharged on the 14th day with positive dynamics. Screening for active CMV infection were was recommended on the month 1, 3, 6, 12 after the discharge from the hospital.

Conclusion. Congenital CMV infection is a poorly diagnosed infection, causing anomalies of the nervous system development. Important aspects are the prevention of the development of CMV in pregnant women, the prevention of its vertical transmission to the fetus, the early detection and treatment of the disease in the newborn.

Particular attention is given today to congenital CMV infection in newborns, which may clinically manifest or have an asymptomatic course. In this regard, it is recommended that all children with sensorineural hearing loss and intrauterine development should be screened for CMV infection (PCR diagnosis of saliva and urine).

Treatment options for congenital CMV infection are limited and include antiviral therapy and passive immunization with specific intravenous immunoglobulin. There are positive results that demonstrate the clinical efficacy of passive immunization with intravenous immunoglobulin containing specific IgG antibodies against the CMV pathogen.

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NUTRITIONAL STATUS AND NUTRITIONAL SUPPORT IN CHILDREN WITH CONGENITAL MALFORMATIONS OF BRAIN IN UKRAINE: SINGLE-CENTER OBSERVATIONAL DESCRIPTIVE CROSS-SECTIONAL STUDY

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Abstract
In Ukraine, as one of the income and middle income countries (LMICs), PEM is detected and diagnosed not quite actively especially in children with neurologic impairment. **Methods:** Nutritive status and energy consumption was evaluated in 17 young and preschool children with congenital malformations of brain by anthropometry, 24-hour dietary recall and questionnaire of caregivers. **Results:** The study demonstrated nutritional disorders: Z-score BW for age in total cohort was –3.2, H/L for age was –2.7. The moderate PEM was diagnosed in 2/17 children, severe PEM in 12/17. The late appointment of nutritional support to such children was demonstrated, its effect on increasing growth and body weight was detected. **Conclusion.** The importance of drawing up individual plans for the energetic consumption of the children with congenital malformations of brain with training of caregivers and rehabilitative and palliative team is advisable.
Keywords: *children, protein-energy malnutrition, congenital malformations of brain, LMICs.*

1. Introduction

Nutrition problems are common in children with neurological impairment [1, 2]. Causes of protein-energy malnutrition (PEM) in them are multifactorial, and malnourishment may be due to motor disorders, digestive problems, medicine use, and the social environment [3].

PEM is one of the most serious medical problems around the world. According to the WHO research, in 22–35% of children from poor families aged 2–6 years, the body weight (BW) is below the 5th percentile, the growth of 11% of children is below the 5th percentile. In hospitalized children, various forms of PEM are still common, which aggravates the course of the disease, worsens their prognosis, and causes a delay in the physical and neurological development of children [4].

In Ukraine, as one of low income and middle income countries (LMICs), PEM is detected and diagnosed not quite actively especially in children with neurologic impairment. Some recent publications demonstrate actuality and research of this problem in other countries such as Bosnia and Herzegovina, Nigeria and Ghana [3, 5, 6].

The main clinical requirements for the assessment of PEM in children is the rapid process of identifying people from the nutritional risk group, which is carried out using developer questionnaires and using appropriate validated scales [7, 8]. The standard method for detecting PEM is the assessment of physical development in children [9].

Assessment of physical development is not always a simple task for children with cerebral palsy (CP) and similar neurodegenerative disorders [3]. Anthropometric evaluation can become a stumbling block in the assessment of the physical development of children with neurological impairment. BW and height or length (H/L), obtained under certain conditions, are often not accurate. Measuring the H/L of lying children is unreliable if the baby has a contracture, high tone of muscles, scoliosis, which interferes with optimal positioning [3, 10]. Despite this, there are more problems in the nutritional status in children

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with neurological disorders. Among them is low awareness of parents and community specialists, low accessibility to clinical nutrition products, due to their high cost, barriers to monitoring experience and nutritive support (NS) among medical personnel as a routine practice in LMICs [6]. Our study was undertaken to assess the nutritional status and nutritional support of children with congenital malformations of brain in Ukraine in order to draw attention to this problem in the community and share our experience.

2. Purposes, subjects and methods:

2.1. Objective: Assessment of nutritional status and nutritional support in children with congenital malformations of the brain.

Hypothesis: nutritional support in children with congenital malformations of the brain has no influence on physical development in children with congenital malformations of brain.

2.2. Subjects & Methods

Study design and setting

A single-center observational descriptive cross-sectional study was performed. It had been carried out in Kharkiv National Medical University and Communal Non-Commercial Enterprise of Kharkiv Regional Council "Regional Specialized Baby Home «Hippocrates»" during 2019. The anthropometric assessment of the children, evaluation of oromotor dysfunction (OMD), a 24-hr dietary recall, assessment of nutritional status before ("baseline") and after 6 months of implementing of food modification ("endline") were studied.

Ethical approval

This study was authorized by the Ethics Committee of Kharkiv National Medical University (Record No. 9 dated 16 October 2018). Informed written consent was obtained from each caregiver in advance of the research. The agreement on implementing food modification (clinical nutrition) was obtained from 9 caregivers.

Sampling

Seventeen children were recruited for the study (young children and pre-schoolers) staying in the department of Palliative Care due to congenital malformations of the brain from May 2019 till October 2019. There were 9 (53%) young children (0–36 months) and 8 (47%) pre-schoolers (3–6 years). The average age was 3.6±2.1 years. Inclusion criteria were children of 0–6 years old with congenital malformations of brain and their caregivers. Children without congenital malformations of brain and their caregivers, children with genetic syndromes and those who did not agree to participate in the study were excluded from the study. All caregivers were females.

Data collection

For nutritional status investigation the anthropometric assessment was used. Anthropometry was measured in accordance with the standard procedure. BW was measured using a digital weighing Infant Scale and was recorded to the nearest decimal place (0.1 kg). The H/L was estimated by means of Infant Length Board marked in cm and nearest 0.1 cm was recorded. For children with paralytic syndromes the H/L was determined by measuring the length of the big tibia (cm) and calculated by formula due to inability to stand, scoliosis or joint contractures of patients.

Body length was calculated as (3.26×length of the big tibia)+30.8 [10, 11]

The BW (kg), and H/L (cm) was carried out in accordance with the Z-scores, using the calculators based on the WHO reference data for infants and children [12, 13]. Z-score curves were used for determination of the severity of PEM following the WHO recommendations (1999) [14].

Individual programs for nutritional support for children and caregivers were proposed as well as training with rehabilitative and palliative team

The anthropometric data and severity of PEM were recorded in two point "baseline" and after 6 months "endline" where nutritional support was carried out.

Additionally the nutritional status included investigation of a 24-hr dietary recall and questionnaire of caregivers. The following questions were included: *1. Does the child usually eat alone or with others? 2. When does the child eat? (Are the meals regular, how many times per day?) 3. Is there sufficient time for feeding? (Does the meal last more or less than 30 minutes?) 4. Do you apply special feeding (If no, what food do you choose?).*

The calculation of the main (basal) metabolism in children (kcal/day) was performed according to Schofield W.N. (1985) by sex, age, BW, H/L taking into account the main conversion factors, as well as determining the true energy needs in "baseline" and after 6 months "endline" [9, 15]. The energy nutritional deficiencies in "baseline" were corrected by food modification (clinical nutrition) during 6 months. The anthropometric data was compared in children with food modification and without food modification in 6 months. We also compared BW and H/L Z-score in children depending on age

(young and preschoolers), and the severity of motor impairment by Gross Motor Function Classification System (GMFCS), and degree of OMD as "mild" degree (eats chopped or mashed food); "moderate" (requires very mushy, chopped or liquid food); "severe" (requires a dense liquid, puree or needs feeding through a tube). The length of daily feeding time (less than 3 hours and more than 3 hours) and meal time (less than 30 min) were estimated [3, 16, 17].

The individual programs of nutritional support for children and caregivers were proposed as well as training by rehabilitative and palliative team.

Analysis

The database for the evaluation of physical development in children was created using Excel for Windows application package (StatSoft Inc.). The analysis of the test results was carried out using standard statistical methods of Statistica 7.0 package: descriptive analysis, difference between two proportions. For nonparametric comparison of independent group Mann-Whitney test (*MW*) was used. In dependent group, the Wilcoxon Rank Sum test (*W*) was used to verify the null hypothesis saying that population in two points, "baseline" and "endline", have the same continuous distribution with power calculation. For all statistical methods, $p<0.05$ was considered statistically significant.

Conflicts of Interest: The authors declare no conflict of interest.

3. Results

3.1 Demographic, Clinical and Feeding Data

Table 1 presents demographic and clinical characteristics of children. The sample included 9 males and 8 females. There were 14/17 children with paralytic syndromes (I–V level of GMFCS). Severe cognitive impairment was established in 8/17. Prevalence of OMD was in total sample, and was distributed as "mild" in 2/17 children, "moderate" in 4/17 and "severe" in 11/17 children. Severe OMD was associated with microcephaly, cognitive impairment and V level of GMFCS.

The feeding time was different in 5 tube-fed children compared with 12 bottle-fed or spoon-fed 12 children (median 11 min vs 32 min). There were no significant differences in correlation of OMD severity, sex or age.

3.2 Anthropometric data, observation of feeding and energy consumption

The results of caregivers' answers for questionnaire demonstrated that all children had meals alone without any social component, regularly, minimum 4 times, maximum 6 times per day. None of them were applied any special feeding formula. The meals length in 4 tube fed children was even less than 15 min. The 24-hr dietary recall demonstrated that only 3 children (younger than 1 year) received formula for feeding, others – "adult" meal (porridges, vegetables, milk and meat, pureed by texture modifications for consistency). All children were unable to feed themselves and needed some feeding assistance.

Table 2 demonstrates individual nutrition status and energy intake for children in "baseline" and "endline" study depending on nutritional support (clinical nutrition). The energy intake in "baseline" means a domestic diet before NS, the energy intake in "endline" was corrected in accordance with the calculation of the basal exchange by Schofield W.N. (1985) taking into account individual child factors (growth and development; PEM; motor activity; impairments as tracheostomy, colostomy, gastrostomy; spasticity; convulsions; rehabilitation) [9, 15].

The moderate PEM was diagnosed in 2/17 children, severe PEM in 12/17 from the total cohort in "baseline" study. The distribution of PEM degree in "endline" was the following: moderate PEM was found in 5/17 children, severe PEM in 9/17.

Our data was collected to compare the children with NS and without NS. The children with NS had much severe deviation of Z-score BW for age at "baseline" (median -6.2 vs -2.1) (MW test $p=0.0111$). We did not find any significant difference in Z-score deviation of H/L for age (median -2.7 vs -34) (MW test $p=0.7429$). The median of Z-score BW for age in total cohort was -3.2 [minimum -0.5 maximum -10.4], of H/L for age was -2.7 [minimum -0.5 maximum -7.1]. Among children who did not receive NS 2/8 children with loss of BW and 1/8 children with loss of H/L. In children who received NS 1/9 children with loss of BW and 4/9 children with loss of H/L. This suggests that a 6-month period with NS for PEM is not enough and requires further monitoring.

We found a significant difference in changes of Z-score BW for age in children under NS during 6 mo "baseline" and "endline" (median -6.2 vs -5.4) (W test $p=0.0208$) and no significant difference in changes of Z-score H/L for age in children under NS during 6 mo "baseline" and "endline" (median -3.4 vs -3.4) (W test $p=1.0$).

To correct PEM in children with congenital malformations of the brain, speech therapists and physical therapists were involved as members of the multidisciplinary team. We proposed the training staff for monitoring the nutritional status in children with PEM and involvement of caregivers.

Table 1

Basic demographic and clinical data

N	Age	Sex	GMFCS	OMD degree	Diagnosis	Comorbid factors
1	7 yrs	M	V	Moderate	Congenital hypoplasia of the cerebellum	Swallowing disorders, contractures, anticonvulsant therapy
2	7 yrs	M	V	Severe	Microcephaly	Sialorrhea, swallowing contractures, cognitive impairment, anticonvulsant therapy, tube feeding,
3	4 yrs 7 mo	F	V	Moderate	Microcephaly	Swallowing disorders, cognitive impairment contractures, anticonvulsant therapy
4	7 yrs	M	V	Severe	Microcephaly	Sialorrhea, swallowing disorders, contractures, cognitive impairment, tube feeding
5	4 yrs 7 mo	M	III	Moderate	Microcephaly	Colostoma, gastrostoma
6	6 yrs 11 mo	F	V	Severe	Congenital hydrocephalus	Sialorrhea, swallowing disorders, contractures, cognitive impairment, anticonvulsant therapy
7	6 yrs 4 mo	F	V	Severe	Microcephaly	Sialorrhea, swallowing disorders, contractures, cognitive impairment anticonvulsant therapy
8	7 yrs	M	V	Severe	Microcephaly	Sialorrhea, swallowing disorders, contractures, cognitive impairment tube feeding, anticonvulsant therapy
9	2 yrs 2 mo	F	V	Severe	Congenital hydrocephalus	Sialorrhea, swallowing disorders, contractures, cognitive impairment, tube feeding, anticonvulsant therapy
10	2 yrs 7 mo	M	IV	Moderate	Congenital hypoplasia of the cerebellum	Swallowing disorders, gastrostomy
11	2 yrs 11 mo	F	I	No	Congenital hydrocephalus	Colostomy
12	6 mo	F	-	Severe	Congenital hydrocephalus	Tracheostomy, swallowing disorders, tube feeding,
13	1 yr 1 mo	F	III	Severe	Microcephaly	Swallowing problem, tube feeding, fetal alcohol syndrome
14	1 yr 4 mo	M	II	Mild	Dandy-Walker malformation	Swallowing disorders, cleft palate, fetal alcohol syndrome, anticonvulsant therapy
15	1 yr 5 mo	M	II	Mild	Microcephaly	Cognitive impairment
16	5 mo	M	-	Severe	Ventriculomegaly	Swallowing disorders, tube feeding, anticonvulsant therapy
17	6 mo	F	-	No	Congenital hydrocephalus	Swallowing disorders

M – male; F – female; yr – year; mo – month; GMFCS – Gross Motor Function Classification System; tube – nasogastric tube.

4. Discussion

The published studies associate neurological impairment in children with PEM [1–3, 6, 9]. PEM is an inadequate nutrition of a child characterized by termination or slowing of increase of body weight, progressive decrease in the subcutaneous basis, violation of body proportions, digestive functions, metabolism, relaxation of specific, non-specific protective forces, propensity to other diseases, delayed physical and psychomotor development [18–20].

Clinical presentation and diagnosis of the PEM are recorded on the basis of an assessment of the physical development of the child by the

Table 2

Z-score of BW and H/L and energy intake in children with congenital malformations of brain during 6 months with and without of nutritional support

N	NS/ NA	Baseline	Endline	Baseline		Endline	
		Kcal/kg day	Kcal/kg day	Z-score			
				BW for age	H/Lt for age	BW for age	H/L for age
1	NA	106	109	-0.5	-4.6	-0.3	-5.0
2	NS	100	134	-2.2	-4.0	-2.0	-3.4
3	NS	116	122	-9.2	-0.9	-7.0	-0.3
4	NS	145	159	-4.2	-5.8	-2.9	-6.0
5	NS	122	171	-6.2	-4.4	-1.6	-4.6
6	NA	104	114	-1.8	-3.2	-2.2	-2.8
7	NS	128	157	-6.3	-0.7	-5.4	-2.1
8	NS	121	122	-10.4	-2.3	-8.7	-1.9
9	NS	112	120	-0.8	-1.7	-0.1	-1.6
10	NS	150	171	-6.4	-7.1	-6.2	-7.4
11	NA	121	111	-3.3	-2.7	-3.4	-2.6
12	NA	110	111	-2.9	-2.7	-2.8	-2.6
13	NS	140	168	-5.0	-3.4	-5.5	-3.5
14	NA	122	118	-3.2	-6.2	-1.5	-5.9
15	NA	105	110	-2.4	-1.2	-1.9	-2.3
16	NA	120	110	-0.5	-0.5	-0.6	-0.5
17	NA	112	117	-1.8	-1.2	-1.8	-1.2

NS-nutritional support, NA-not applicable.

Z-score. Insufficient BW or H/L is recorded according to the Z-score interval between -2 and -3 for the given age. Excessively insufficient BW or H/L is detected when the Z-score range is below -3 for the given age [13, 14]. We have also investigated PEM in children with neurological impairments. More publications have shown PEM in children with CP [3, 5, 6, 10, 11, 16, 21].

According to the review of Francesca Penagini et al., among 16 publications on dietary intakes and nutritional issues in neurologically impaired children, 10 publications show the results concerning NS in children with CP, others with disabilities and delay in motor development [2]. Despite the fact that the problem of nutritive insufficiency in children with neurological impairments is being actively studied, the results of the studies differ [2].

Our study is original due to recruitment of children with congenital malformations of the brain. Furthermore, undernourishment in children with neurological impairment remains one of the major challenges to the health system for LMICs. Some publications demonstrated similar problem [3, 5, 6, 22]. They have estimated that up to 200 million children are not reaching their development potential in LMICs [22, 23].

Our study demonstrates that growth trajectories in Ukrainian children with neurological impairment and undernourishment also depend on the knowledge level of the caregivers and medical staff due to loss of education and are similar to those obtained by Claudia Mary Donkor in Ghana [6].

A big step forward for our staff and our Non-English speaking country was to create the WHO a software "Anthro" in Russian. The results of our study influenced teaching of approaches to anthropometry by the staff and caregivers, time of feeding, energy needs and possibility to apply special nutrition which implies an informational and educational company in the community [6].

Moreover, our results show the issue of gastrostomy in children on local level [24]. These challenges mirror the reports of previous studies in similar patients [25].

The next special issue for discussion is anthropometry. The use of National Center for Health Statistics (US) for anthropometry of children with CP has been used in a large number of studies [26]. According to the review of Srishti Aggraval et al. only one study from Bangladesh applied the WHO standards for growth of children with CP [26, 27]. The study included 37 children 1–11 yrs old with moderate and severe CP. The mean Z-score for BW was -4.83±1.84, mean Z-score for H/L was -2.7±1.98. We compared our total cohort, the mean of Z-score for BW was -3.94±2.93, and mean Z-score for H/L was -3.12±1.98 (*Table 2*). Our cohort was dominated

by children with shunting. This indicates a more severe form of PEM and late attention to this problem in our children.

Energy consumption was calculated in 90 children age 2–13 yrs old according to the type of CP (hemiplegia, diplegia, and tetraplegia) by Patricia Ayrosa C. Lopes et al. [28]. Similarly, to our study, the authors used the 24-hour recall method and a questionnaire of caregivers to calculate daily calorie intake, with the subsequent recommendation of its correction by the American Food Guide, we used the recommendations of ESPGHAN applying Schofield W.N. formula (1985) and conversing factors [9, 15]. Energy intake in Patricia Ayrosa C.Lopes et al. study was showed according to the age. We suppose that calories must be calculated in accordance with BW because children with neurological impairments have delay of BW.

Our results are also similar to those obtained by most researchers about the problems of swallowing in children with neurological impairments, and therefore, their meal time is lengthened [3, 6, 28, 29]. But we found that tube-fed children had catastrophically shorter feeding time, which could lead to complications [30]. We suggest that there is a need in special studies for the same patients.

One of the most important issues regarding nutrition management in children with congenital malformations of brain is to apply NS. Just like the study, in which forty-five young patients aged between 2 and 26 years with severe neurologic impairment (GMFCS level V) were recruited, were identified with moderate or severe malnutrition, including the patients who received an intervention during a 6-month period, we have shown an improvement of nutritional status at the same period in our sample [31]. The difference was in the type of NS (gastrostoma vs nasogastric tube or orally). Whether the children received nutrition through a nasogastric tube or orally, for 6 months of nutritional support, we received positive results due to availability and utilization of nutrients from enteral formula, which was significantly better than the food prepared by the caregivers.

There were some inherent **limitations** associated with this study; firstly, sample size. Our model was based on single-center observational descriptive cross-sectional study and was limited by the time and number of patients in the East Ukrainian population with congenital malformations of the brain. Secondly, there was an age limitation (young children and preschoolers). There are very few prior researches and gaps in the studies relevant to young children with congenital malformations of the brain, which influenced the methodology of our study. Our study was limited by the recruitment of infants, which was not completely appropriate for GMFCS. We were unable to assess each factor on malnourishment, which may undermine the strength of the study.

Conclusion

The study demonstrated moderate and severe nutritional disorders in young children and pre-schoolers with congenital malformations of the brain: Z-score BW for age in total cohort was -3.2, H/L for age was -2.7 in LMICs. Its results differ from the previous studies that include children with CP. Late appointment of nutritional support to such children was demonstrated, as well as its effect on increasing growth and body weight. The importance of drawing up individual plans for the energetic consumption of the children with congenital malformations of brain with training of caregivers and rehabilitative and palliative team was shown. High-quality clinical trials are needed to better comprehend the methodology of nutritive support in children of any age with different neurological impairments.

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ROLE OF B-DEFENSINS IN IMMUNE RESPONSE IN TUBERCULOSIS PATIENTS

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Abstract
Expanding of tuberculosis drug-resistance makes host-directed treatment an important part of tuberculosis treatment. Host-directed treatment is aimed at stimulating the production of antimicrobial peptides by the patient's immune cells. The use of β-defensins is very interesting in this field because of their pronounced bactericidal and bacteriostatic effects, as well as the ability to stimulate the chemotaxis of immune cells. The article presents a review on the immunological properties of the defensin family and the possibility of their use in practice. To complete the review 114 articles from "PubMed" resource were analyzed, 34 of them were chosen to review immunomodulatory and antimicrobial action of β-defensins.
The original research results on Human-beta-defensine-1 use as tuberculosis severity marker were also added to the research. To obtain our own research results, 100 TB patients and 20 healthy persons were included in the study. Human-beta-defensin-1 level in serum was investigated in all the patients at the treatment onset and in healthy persons. Mann-Whitney U test (for comparison of 2 independent groups) and Spearman's rank correlation coefficient were used for statistical data processing. It was found that Human-beta-defensin-1 level was significantly higher in TB patients than in healthy persons. A correlation of medium strength ($r=+0.53$, $p<0.05$) between Human-beta-defensin-1 and tuberculosis lesion volume was revealed. The data obtained allows using human β-defensin-1 as a diagnostic marker of tuberculosis.
Key words: *tuberculosis, β-defensins, immunity, prognostic marker.*

1. Introduction

According to the World Health Organization (WHO), tuberculosis is among the 10 most common causes of death in the world. In 2019, about 1,600,000 deaths from tuberculosis were recorded and about 10,000,000 new cases were detected, which corresponds to an incidence rate of 133 per 100,000 population [1].

According to the WHO objectives within the "End TB" program, it is necessary to reduce the incidence of tuberculosis by 90% and reduce the mortality rate by 95% to overcome the tuberculosis epidemic. For this, it is necessary to increase the susceptible tuberculosis treatment effectiveness up to 85% and to increase multidrug-resistant tuberculosis treatment effectiveness up to 75% [2].

One of the main factors affecting the course of tuberculous process and the treatment effectiveness is severity of tuberculous lesions and the ability of the immune system to provide an adequate response. In this section, it is interesting to study the cationic peptides of the immune system, which have multi-profile functioning in the immune response: immunomodulating activity, direct antimicrobial action, selective inflammatory and anti-inflammatory properties and additional reparative activity [3].

Among the cationic peptides of the immune system, a family of defensins is distinguished. Defensins are cysteine-containing peptides consisting of 29–35 amino acid residues. They are able to form 3 invariant disulfide bonds, which determine the distribution of defensins into 3 subgroups – α, β and θ [4, 30].

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2. Purposes, subjects and methods:

2.1. The **aim** of the study was to review the available data and find out the immunological role of β-defensins and their possible practical role in the diagnosis, prediction and treatment of tuberculosis.

2.2. Subjects & Methods

114 articles from "PubMed" resource were analyzed, 34 of them were chosen to review immunomodulatory and antimicrobial action of β-defensins. To obtain our own research results, 100 TB patients and 20 healthy persons were included in the study. Human-beta-defensin-1 level in serum was investigated in all the patients at the treatment onset and in healthy persons. Mann-Whitney U test (for comparison of 2 independent groups) and Spearman's rank correlation coefficient were used for statistical data processing.

Conflict of interests. Authors have no conflict of interests.

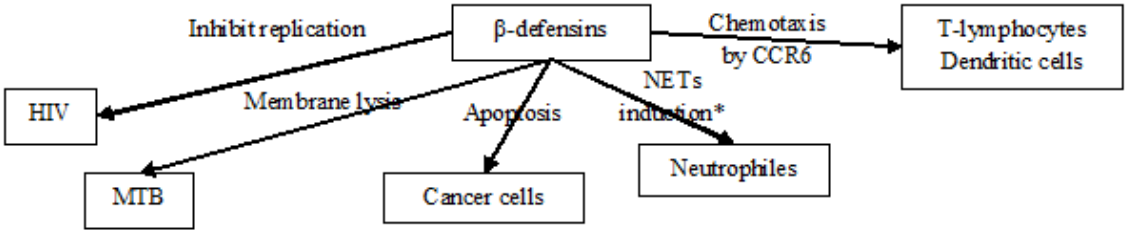
3. Results

The defensins, namely β-defensins, were first isolated from human blood by Lehrer et al. in 1985 [17]. However, β-defensins subgroup is the most interesting among defensins, as they are produced in majority of the organs and body systems, including bronchial epithelial cells, neutrophils (azurophilic neutrophil granules), NK-cells and certain types T-lymphocytes [5, 25, 28]. Their expression is stimulated primarily by the influence of TNF-α, IL-1, in response to the recognition of bacteria by Toll-like receptor-mediated pathways, as well as by the direct action of bacterial agents. It makes them potentially sensitive markers of the disease severity [6, 29].

β-defensins exhibit chemotactic activity and activate migration of T-lymphocytes, macrophages, dendritic cells [7, 34]. The expression of β-defensins is activated directly under the influence of *M. tuberculosis* (MTB). The production of β-defensins by some cells triggers its production by others [8–9].

In addition, β-defensins exhibit direct antimicrobial activity by damaging the cell membranes of bacteria that have been captured by phagocyte or persist in extracellular matrix [10]. After penetration into the microbial cell, β-defensins can bind polyanionic DNA molecules [11]. The main immune role of β-defensins is shown in *Fig. 1*.

Some studies have already determined the role of β-defensins as markers of tuberculous lesion severity [12, 22], as well as markers of the transition of latent tuberculosis infection to the active phase [23]. In addition, defensins can act as a marker of treatment efficacy, since their level probably progressively decreases to normal value while recovery and achieving a positive effect of treatment [13, 21].



* *Neutrophilic extracellular traps (NETs) are networks of extracellular fibers, primarily composed of DNA from neutrophils, which bind pathogens*

Fig. 1. Immune action of β-defensins

A decrease in the level of β-defensins was also found in patients with severe comorbidities, such as diabetes mellitus, accompanied by a greater severity of tuberculosis lesions and slower healing [26]. In a study by Zhu et al., 2011, a correlation between the low level of defensins and the development of multidrug-resistant tuberculosis was suggested [27].

The listed properties of β-defensins also determine their promising role in improving the pathogenetic treatment of tuberculosis, the so-called host-directed therapy [31]. In a study by A. Kalita, 2004, a synergistic effect of β-defensins and anti-tuberculosis therapy was observed, since β-defensins damage membranes of *M. tuberculosis* and facilitate the penetration of anti-tuberculosis drugs into the cells [14], and also have a direct effect on pathogens located intracellularly [9]. In another study, the bactericidal activity of neutrophils that do not contain defensins and neutrophils that contain them was experimentally compared, and it turned out that the family of defensins plays a key role in the

destruction of mycobacteria [25]. The study by Rivas-Santiago et al., 2008, showed the bacteriostatic role of β-defensins and their ability to prevent the reactivation of latent mycobacteria by binding *M. tuberculosis* DNA [28]. The study by Sharma et al. found the minimum concentration of β-defensins-1 in vitro (2 mg / ml), which inhibits the intracellular growth of *M. tuberculosis*. Comparison of the action of β-defensins and Rifampicin in vitro showed a high inhibitory activity of the antimicrobial peptide [32].

Artificial administration of recombinant defensins is not reliable due to the high cost and short half-life of the peptides. This idea is under development, but recombinant β-defensins demonstrate bactericidal activity against chemoresistant *M. tuberculosis* strains in vitro [23]. However, a possible solution is to stimulate the production of defensins by the body's own cells. In 2011 and 2015, Rivas-Santiago et al. demonstrated in experimental animal models (research in mice) that the use of L-isoleucine stimulates the production of its own β-defensins and reduces bacterial excretion and the severity of infiltrative tuberculosis lesions [15–16]. Later in 2013, a similar study was performed on pigs and showed that the use of L-isoleucine in combination with zinc intensifies biosynthesis of β-defensins and stimulates the macrophage immune response [18]. The same results were obtained when studying the cell model [33].

The study of the mechanism of L-isoleucine action in the culture of epithelial cells suggested that the stimulation of β-defensins production occurs by an intracellular effect on the chiral receptor or enzyme by the activation of transcription factors of the NF-kByrel family [20].

Increased immune response may be associated with an increase in the synthesis of β-defensins under the influence of L-isoleucine and by CCR6-mediated chemotaxis of CD4 + T-lymphocytes stimulated by influence of isoleucine and leucine in the N-terminal region of the α-helix of β-defensins [19].

The original research results

Human-beta-defensin-1 level was 21.48±2.88 U/L in TB patients and 8.97±2.56 U/L in healthy persons (*Fig. 2*). The differences between the groups were significant ($p<0.05$). A correlation of medium strength ($r=+0.53$, $p<0.05$) between

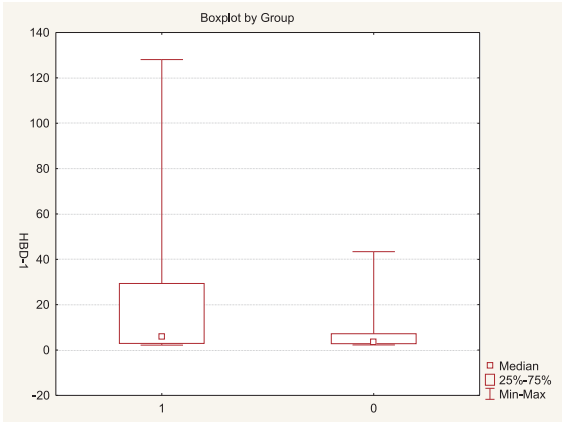


Fig. 2. Differences in Human-beta-defensin-1 level in TB patients (Group 1) and healthy controls (Group 0)

Human-beta-defensin-1 and tuberculosis lesion volume was revealed.

4. Conclusions

The determination of β-defensins level can predict severity of tuberculosis and the effectiveness of anti-tuberculosis therapy. Secondly, β-defensins, due to pronounced bactericidal and bacteriostatic effects, as well as the ability to stimulate the chemotaxis of immune cells, can be used to increase the effectiveness of anti-tuberculosis therapy. However, the use of recombinant β-defensins is associated with difficulties in their synthesis and the instability of peptides artificially introduced into the body. Therefore, the prospect of improving the pathogenetic therapy of tuberculosis is to stimulate the production of defensins by the human cells by the use of L-isoleucine. Study of human β-defensin-1 in tuberculosis patients of our hospital revealed positive correlation of medium strength between human β-defensin-1 level in serum and volume of tuberculosis lesions which allows using human β-defensin-1 as a diagnostic marker of tuberculosis.

List of abbreviations:

NK – Natural killers

TB – Tuberculosis

WHO – World Health Organization

Conflict of interests

The authors declare that they have no competing interests.

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