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CUPRINS

EXOSOMES AS VALUABLE PLAYERS IN HEALTH AND DISEASES

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ABSTRACT

Normal human physiology involves a well-coordinated cross-talk between different cell types. Throughout evolution, multicellular organisms have developed a variety of signalling pathways in the local microenvironment, as well as distant sites. Besides hormones and protein-protein interactions, exosomes provide an alternative communication strategy with high specificity and stability and increased penetrability in various organs and tissues due to their low immunogenicity profile and reduced size.

In this review we will focus on exosome biogenesis, their role in coordinating various physiological and pathological pathways, different strategies of exosomal isolation, as well as potential future applications in early diagnostic and drug delivery.

Key words: exosome, inflammation, metastasis, angiogenesis, cell differentiation, tumor microenvironment

INTRODUCTION

Microvesicles are small, lipid-enclosed vesicles that are released into the extracellular space to facilitate intercellular communication. Apart from apoptotic bodies, ectosomes and endosomes exert pleiotropic effects, being involved in complex processes such as angiogenesis, cell differentiation, adhesion and inflammation [1-3].

Microvesicles transport various biomolecules such as lipids, proteins or nucleic acids. Exosomal transmembrane and surface proteins facilitate protein-protein interactions from distant cells. Specific immune response relies on antigen-presenting cell-derived exosomes containing MHC class I and class II molecules, which aid in early pathogen recognition [4-5].

Immunosuppressive and angiogenic exosomes express PD-L1 or VEGF, favoring the formation of a hospitable tumor microenvironment before the arrival of cancer cells in the process of metastasis [6].

Nucleic acids found in the exosomal cargo include microRNAs (miRNAs) and messenger RNAs (mRNAs) which alter the expression of target genes and hence, modulate cellular signaling pathways involved in growth, differentiation, adhesion and migration [7].

Similar to liposomes, specific cell-derived exosomes can be loaded with drugs in order to improve their pharmacokinetics. However, drug loading techniques involve a sufficient number of exosomes with intact biological functions [8]. A large and variate palette of isolation techniques exists in the actual research field and each technique has its own advantages and disadvantages [9]. Further research is required to improve the yield of exosome isolation and drug internalization. Finally, drugs that were once discarded due to their poor pharmacological properties can be reconsidered by encapsulating them into extracellular vesicles.

MICROVESICLES NOMENCLATURE

Microvesicles released in the extracellular milieu are divided into three categories based on their size and cellular activity: exosomes, shedding microvesicles and apoptotic blebs [1, 10].

Shedding microvesicles (SMVs) or ectosomes are large vesicles (100-1000 nm) released by plasma membrane budding. The process of budding is mediated by proteins such as calpain, flippase, floppase, scramblase and gelsolin [1]. Compared to exosomes, SMV cargo does not undergo prior selection before entering the lumen.

Apoptotic blebs are platelet-sized vesicles (approximately 500 nm) released into the extracellular space by cells undergoing programmed cell death. ABs are phagocytized by neighboring cells and their contents are used in further biomolecule synthesis. Therefore, they are not involved in intercellular communication [10].

Exosomes are extracellular vesicles with origin in

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the endolysosomal compartment [10] and display different biogenesis, release pathway, size (30-150 nm), content and function than SMVs and Abs [7] (Figure 1).

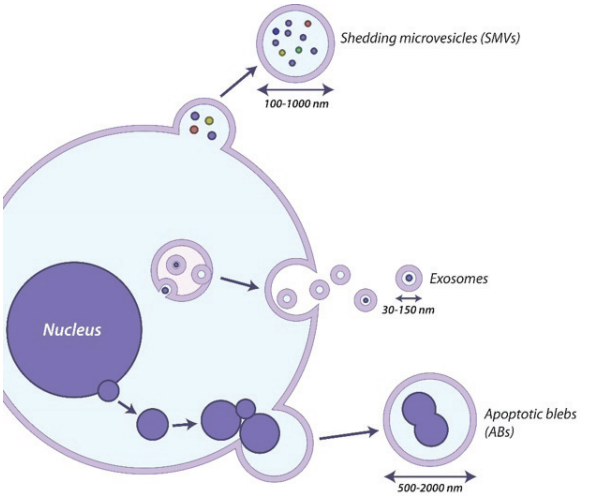

Fig. 1. Shedding microvesicles are released by the outward budding of the plasma membrane. Exosomes are produced by inward budding of the multivesicular body and subsequent fusion with the plasma membrane. Apoptotic blebs are released when cells undergo programmed cell death and contain condensed nuclear material and degraded organelles.

BIOGENESIS OF EXOSOMES

Exosomal production starts with the process of plasma membrane invagination forming an early endosome. The endosomal membrane suffers an inward budding (into the lumen), creating membrane-bound vesicles in a structure called multivesicular body (MVB). The vesicles are then separated from the membrane in a twisting motion resulting intraluminal vesicles (ILVs). The last step involves the fusion of the plasma membrane with the MVBs and the release of ILVs into the extracellular space. The zipper-like fusion process is mediated by the interaction between v-SNARE (present on the MVB) and t-SNARE (the target plasma membrane) forming the SNARE complex [11].

The classical pathway for exosome formation depends on the ESCRT (endosomal sorting complexes required for transport) multimeric protein complex. ESCRT comprises 4 protein complexes with specific, well-coordinated functions. ESCRT0 recruits the proteins that will be translocated into the intraluminal vesicle. In the classical pathway, ubiquitinylated proteins enter in the intraluminal space [1]. ESCRT-1 and ESCRT-2 are the main effectors of membrane budding and ILV formation in the intracellular compartment, while ESCRT-3 and the VPS4 ATP-ase cleave the membrane, releasing the membrane-bound vesicle into the intraluminal space. ESCRT-independent exosome biogenesis depends on the tetraspanin protein family which induces membrane curvature and allows vesicle formation. Members of the tetraspanin family such as CD9, CD81 or CD63 can be used as markers for exosomal isolation in immune-affinity assays [10,12].

Membrane curvature and budding is also facilitated by certain lipids present in the exosomal membrane - ceramides, cholesterol and extracelullar facing phosphatidyl-serine [6].

Compared to proteins that are secreted in the Golgi apparatus or in the ESCRT-dependent pathway that require specific signaling sequences, ESCRT-independent proteins do not obey known rules. Ras-Carmona et al. developed a random-forest-based algorithm that predicts proteins that are more likely to be included in exosomes, allowing further biomarker discovery [13] (Figure 2).

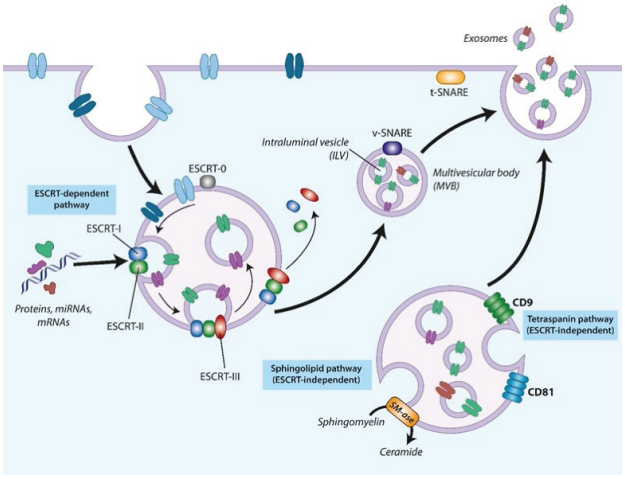

Fig. 2. Exosome biogenesis starts with plasma invagination (top left) and creates an early endosome. In the ESCRT-pathway, the ESCRT protein complex mediates the intraluminal vesicle formation (ILV) and protein and nucleic acid enrichment inside the ILVs. The multivesicular body (MVB) fuses with the plasma membrane in a SNARE-mediated fashion, releasing the exosomes in the extracellular space. The ESCRT-independent release involves ceramide produced by sphingomyelinase (SM-ase) or tetraspanins (CD9, CD81, CD63) to induce the membrane curvature.

Exosomes influence the target cells in four ways. The first mechanism involves the direct protein-protein interaction between the exosomal proteins and the target cell. In the second mechanism, the surface proteins are cleaved by extracellular proteases, exposing different binding sites that can interact with the target cell. Direct fusion of the cell membrane with the exosomal particle facilitates miRNA and mRNA transfer inside the cytoplasm. The last pathway consists of internalization of the exosome via endocytosis or phagocytosis [10].

BIOLOGICAL FUNCTIONS OF EXOSOMES

Antigen presentation and lymphatic trafficking

Srinivasan *et al.* showed that exosomes containing foreign antigens are rapidly (in a matter of minutes) transported from

the infection site into the lymphatic system. Regional lymphatic vessels express high permeability for exosomal particles facilitating translocation into the lymphatic vessel lumen.

Extracellular vesicles released during the inflammatory response originate from resident antigen-presenting cells [10] and from normal infected cells. Exosome release in the lymphatic stream allows rapid flow of information that precedes the arrival of effector cells at the infection site [5].

Once reaching the lymph node, the exosomes are uptaken by B-cells and macrophages which prime the helper and cytotoxic T cells resulting in a prompt and specific immune response.

T-cell-derived exosomes express TCR which in turn trigger interferon-gamma and granzyme production in naive CD8+ T cells, leading to an inflammatory positive feedback [7].

This phenomenon was demonstrated in mice infected with M. tuberculosis that expressed high levels of infected macrophages-exosomes containing mycobacterial lipoproteins [4].

The lymph node route is also exploited by melanomas, tumors characterized by initial lymphatic dissemination. Once in the lymphatic system, melanoma-derived exosomes induce an immunosuppressive environment allowing subsequent lymphatic invasion. Recent work showed that B16F10 melanoma cell-derived exosomes reached the subcapsular sinus macrophages and inhibited their anti-tumor functions [6].

Immune regulation and pregnancy

Pregnancy involves a tightly regulated bilateral communication between the fetal and maternal organisms mediated by hormones, cytokines and exosomes which involve fetal vasculogenesis. Dysregulation in exosome composition or number are associated with gestational-related pathologies such as pre-eclampsia, gestation diabetes or fetal growth restriction [14].

Exosomes produced by fetal cells interact with specific maternal cells to induce maternal changes required to sustain fetal growth. Placental-derived extracellular vesicles express immunomodulatory molecules such as PD-L1 or CD276 that prevent an undesired cellular immune response against the fetus.

MiRNAs miR-516b-5p, miR-517-5p, and miR-518a-3p present in placental-derived exosomes modulate the PI3K-Akt and the insulin signaling pathways, suggesting their critical role in gestational diabetes or intra-uterine fetal growth restriction. Endometrial-derived exosomes contain hsa-miR-30d, a micro-RNA that enhances integrin subunits α7, β3 and cadherin-5, facilitating trophoblast invasion and adhesion. Changes in the spiral artery microarchitecture involve hypertrophy, hyperplasia, apoptosis and extracellular membrane remodelling, all mediated by extracellular vesicles released by the cytotrophoblast [15]. Hypoxic conditions stimulate the release of VEGF-containing extracellular vesicles by the placenta, leading to increased blood flow and angiogenesis. HUVEC cell line, treated with exosomes isolated from the plasma of pregnant women stimulated proliferation, migration and angiogenesis [16].

Exosomes modulate the tumor microenvironment architecture and facilitate tumor metastasis

The tumor micro-environment is an intricate biological milieu that involves a complex communication between tumor and normal resident cells, such as fibroblasts, endothelial cells or immune cells [17]. By hijacking the physiological anti-tumor immune response, angiogenesis and wound healing processes, malignant cells assure their survival, proliferation and invasion in regional or distant sites [18].

Besides cytokine release, cancer cells communicate through extracellular vesicles that contain various bioactive molecules. Nucleic acids such as micro-RNAs or small interfering RNAs can alter the expression of tumor suppressors in target cells, leading to abnormal cellular proliferation. Proteins involved in cell signaling such as growth factor receptors can integrate into the plasma membrane of the target cell, allowing increased sensitivity to growth factors [1,6].

Recent studies have shown that enzymes involved in adenosine metabolism are highly expressed on the tumor-derived exosomal surface. Molecules such as CD73 and CD39 hydrolyze adenosine triphosphate to adenosine in the tumor microenvironment. High concentrations of adenosine interact with adenosine receptors A_{2A} and A_{2B}, limiting the proliferation of cytotoxic T cells [7,19,20]. High expression of CD73 and CD39 is associated with a higher stage malignancy (III/IV) and can be used as a future biomarker to predict prognosis [19].

Tumor-associated exosomes can pass into the bloodstream and allow cellular communication at distant sites. Signals emitted by tumor cells favor the development of a pre-metastatic niche, a cluster of different non-tumoral cells present in a target organ for metastasis (liver, lung, bone etc.), that creates a hospitable, immunosuppressive, highly vascularized microenvironment. By creating the pre-metastatic niche, the tumor educates the target organ how to prepare for its arrival.

Pre-metastatic niche formation is a well-coordinated multi-step phenomenon that involves several crucial steps. Tumor growth, invasion and metastasis are highly dependent on the nutrients provided by blood flow. Pro-angiogenic extracellular vesicles contain high concentrations of cytokines such as VEGF (vascular endothelial growth factor) or long-noncoding RNAs such as aHIF (antisense hypoxia-inducible factor) [21]. Tumor-derived proangiogenic vesicles can also function as a cellular disposal mechanism, contributing to drug resistance in targeted cancer therapy with VEGF inhibitors [10,21].

Local invasion and angiogenesis rely on extracellular membrane remodelling. Pancreatic cancer cells release in the portal circulation exosomes loaded with macrophage migration inhibitory factor (MIF). MIF induces TGF-β secretion from Kupffer cells, which in turn stimulates fibronectin production by stellate cells in the perisinusoidal spaces of the liver [6]. Peritoneal metastasis is promoted by exosomes containing MMP-1 (matrix metalloproteinase 1) secreted by ovarian cancer cells in mice [22].

The immunosuppressive tumor microenvironment relies upon the secretion of exosomes. Tumor-derived exosomes

induce differentiation of monocyte and M1 macrophages to an anti-inflammatory phenotype. TGF-**β**, IL-10 and other cytokines reduce the local activity of anti-tumoral T-lymphocytes. Additionally, several subpopulations of exosomes express the pro-apoptotic molecules FasL and PD-L_1. Cytotoxic T cell response is also hampered by the increasing number of regulatory T cells and BMDCs (bone-marrow derived dendritic cells).

Tumor metastasis organotropism depends on the adhesion molecules expression pattern on exosomes. Tumor-derived exosomal cargo expresses different integrin patterns which decide the organs in which the pre-metastatic niche forms and metastasis occurs. Relevant examples include $\alpha_6\beta_4$ and $\alpha_6\beta_1$ integrins in breast cancer with pulmonary metastasis or $\alpha_v\beta_5$ integrin enriched exosomes in hepatic metastases of pancreatic adenocarcinomas. The integrin patterns on TEVs (tumor extracellular vesicles) are highly organ-specific and may predict future organ metastasis in cancer patients [6].

Endometriosis

Endometriosis is a chronic inflammatory disease characterized by the presence of ectopic hormone-dependent endometrial-like tissue containing glands and stoma, leading to pain and infertility. Freger *et al.* demonstrated that miRNAs, lncRNAs and proteins contained within the exosomes contribute to the characteristic cell signaling malfunctions – angiogenesis, chronic inflammation, invasion, migration, proliferation and apoptosis [14].

Greening *et al.* showed that endometriosis cell-derived exosomes induce fundamental changes in normal trophoblastic cells involving adhesion capacity by modulating the FAK (focal adhesion kinase) pathway [23].

Endometriosis involves a complex cross-talk between stromal-ectopic cells and M2-macrophages. The presence of ectopic endometrial tissue in target organs (ovary, peritoneum, utero-sacral ligaments) modulates the differentiation of circulating monocytes to a M2 phenotype with subsequent release of immunosuppressive cytokines (IL-10, TGF-β) that allows ectopic tissue implantation [14].

One mechanism proposed for extrauterine endometrial invasion involves retrograde menstrual cell flow into the pelvic area. Along with menstrual cells, exosomes that pass in a retrograde fashion can prime the ectopic situs for implantation by enhancing angiogenesis. Endometriosis lesions express high vascularization, dependent on the menstrual cycle [24].

Another mechanism that can explain endometriosis developed in women with Rokitansky-Mayer-Kuster-Haster disease involves coelomic metaplasia, a process in which cells that line the peritoneal or pleural cavities transdifferentiate into functional endometrium. The process of metaplasia might involve miRNAs, mRNAs or regulatory proteins present in the exosomal cargo.

The angiogenic-related lncRNA aHIF (antisense hypoxia-inducible factor) was found in high concentrations in ectopic endometrial cells as well as endometriosis patients' sera. Supporting evidence show that HUVEC treated with endometriosis-derived exosomes showed high VEGF release and b-FGF, suggesting enhanced angiogenesis [25].

Diabetes and cardiovascular diseases

Diabetes mellitus represents an important risk factor for cardiovascular disease development due to the pro-inflammatory and hormonal imbalance that leads to atheroma plaque formation. Extracellular vesicles identified in the sera of DM patients provide additional information about the interconnection the role of DM in atherosclerosis biology.

The main actors involved in atherosclerosis are the endothelial cells, vascular smooth muscle cells and monocytes/macrophages. Endothelial dysfunction stimulates the release of IL-6 and IL-8-containing extracellular vesicles that promote monocyte migration to the subintimal layer. Extracellular vesicles also promote cell adhesion by enhancing expression of ICAM-1 in endothelial cells, as well as CD11b in monocytes. Moreover, microRNAs such as miR-10-a promote NF-KB signaling pathway in monocytes leading to a local pro-inflammatory state [10].

Vascular homeostasis implies the existence of a cross-talk between the endothelial cells and the vascular smooth muscle cells. Shear stress that acts on the endothelial surface provides a stimulus for changes in vascular tone [26]. Heo *et al.* demonstrated that in arterial injury, exosomes released by endothelial cells and platelets contain PDGF (platelet-derived growth factor) which induces phenotypic changes in vascular smooth muscle cells – from a contractile phenotype to a collagen-secreting one. Moreover, the miRNA repertoire of VSMCs altered the endothelial biology, enhancing EC proliferation and migration [27].

Adipocyte-derived exosomes from obese patients act in an endocrine fashion on macrophages, potentiating their inflammatory response. Paracrine signaling on neighboring adipocytes impairs glucose uptake and promotes insulin-resistance and altered response to glucose.

The link between obesity, diabetes and atherosclerosis can be found in the insulin-resistant adipose cell exosomes. Molecules such as sonic the hedgehog protein enhance the expression of pro-inflammatory cytokines (TNF-alpha, IL-1beta, IL-6) and adhesion molecules (ICAM-1), promote vascular remodeling via MMP2 and MMP9 in the atheroma plaque and promote vasa vasorum angiogenesis (VEGF-A) [10].

Serum biomarkers used in diabetes (glycemia, glycated hemoglobin) suffer deviations from the normal values only in clinically manifest disease. Because of their biomolecular similarity with the parental cell, exosomes reflect cellular alterations before the disease onset. Extracellular vesicles can be isolated from any extracellular fluid (blood, urine, cerebrospinal fluid, peritoneal tap etc.) and can predict the individual risk for developing a certain pathology or complication [1,8].

EXOSOME ISOLATION AND ANALYSIS PROCEDURES

Ultracentrifugation

Ultracentrifugation is considered the classical method for isolating exosomes. [6] After a low-speed centrifugation to remove all the cells and debris from the suspension, a variable number of centrifugation cycles with increasing rotational force and cycle time will make smaller particles to pellet sequentially. When reaching 100,000-120,000 x g, exosomes can be isolated from the suspension.

The disadvantages of this procedure include protein and lipoprotein contamination, vesicle aggregation which leads to a lower yield [3] and high mechanical stress due to high centrifugal forces that may alter exosomal physical and biological properties. Exosomes isolated using ultracentrifugation alone cannot be used for drug delivery systems or functional analysis [9].

Gradient-density ultracentrifugation

Derived from blood cell separation techniques, gradient-density centrifugation exploits the physical property of particles with a specific density to remain suspended in the fluid region with the same density.

The density gradient is created using various concentrations of a biocompatible solution such as dextrose or iodoxinol. The sample of interest is added to the tube and centrifuged for 16 h at 100,000 x g. After the ultracentrifugation step, all the components migrate to their corresponding density zone and reach isopycnic position.

Compared to ultracentrifugation alone, density-gradient ultracentrifugation provides a better separation of extracellular vesicles from protein aggregates, mainly because the proteins are denser. Additionally, the density gradient provides a cushion for mechanical stress, maintaining the EV structure and biological function intact [3]. However, it cannot isolate strictly the exosomes, because certain microvesicles with different sizes and composition may show an equal density (1.1-1.21 g/ ml) [1,9].

To overcome this problem, a moving-zone density separation is required. This process involves a solution with a density significantly lower than of the particles that need to be separated. After centrifugation, all the suspended particles will begin to sink proportionally to their mass/size, allowing further vesicle separation [9].

Size-exclusion chromatography

By passing an aqueous solution through a porous stationary phase, molecules behave differently based on their size. Smaller molecules tend to pass through the pores and are slowed down by their longer traffic distance. Larger molecules that cannot fit inside the pores are forced around the porous particles and released faster. Therefore, by collecting the particles that pass through the chromatography column at specific time intervals, a higher yield exosome isolation is obtained.

Because SEC is performed by passive gravity flow, it does not affect the EVs integrity and preserves their biological functions [9].

Precipitation

Precipitation utilizes highly hydrophilic polymers, such as PEG (polyethylene glycol) [12] that interact with the water molecules that surround the exosomes. PEG-water interactions create a peri-exosomal hydrophobic micro-environment which favors their precipitation.

The drawback of this method is that PEG precipitates not only exosomes, but also other biomolecules, such as proteins and nucleic acids. The solution to this inconvenient is an ATPS (aqueous two-phase system) consisting of a denser, more hydrophilic dextran solution and a PEG solution. After a low-speed centrifugation cycle, the exosomes migrate in the dextran phase, while the proteins and nucleic acids migrate in the PEG phase [9,12].

Immune-based assays

Monoclonal antibodies have high specificity for their target protein and have been largely used in clinical applications and research. Flow cytometry exosome isolation involves antibodies linked to fluorescent molecules and require a lower detection threshold due to exosomal significantly reduced diameter.

Immune-based assays provide a high yield exosome separation with the possibility to isolate certain exosomal sub-populations [12]. Exosomes express non-specific markers that aid in basic exosomal isolation. These markers include: LAMP2B (lyososme associated membrane protein 2B), heat shock proteins, PDGF receptors, flotilin, Rab5, CD81, CD82, CD63, CD9, and Alix [10].

By detecting markers originating from their parental cells, exosomes can further be divided into subpopulations. Examples include EPCAM and EGFR for early cancer detection [1,9].

Immunological assays can only be used for diagnostic purposes because of their non-neutral pH and non-physiological elution buffers (for separating exosomes from the antibodies) that affect irreversibly the biological function of the collected exosomes [9].

Tim-4-based isolation

In 2016, Nakai *et al.* developed a high-yield, non-immunological exosome isolation based on the interaction between the protein Tim-4 and the phosphatidyl-serine molecules present on the exosomal surface. This Ca^{2+}-dependent interaction can be reversed by adding a calcium chelating agent such as EDTA (ethylene-diamino tetraacetic acid) allowing exosome recovery with intact biological function [28].

Microfluidics

Microfluidics-based approaches provide an attractive alternative to classic exosome separation techniques due to their high yield, low amount of patient sample (several microliters) and short isolation time (several minutes, compared to the 16 h ultracentrifugation [9,23]), allowing future bed-side diagnostic tools.

Immunoaffinity-based microfluidics involve passing a

laminar flow of sample into chip channels coated with exosome-specific antibodies (anti-CD63, CD9, CD81). To increase the probability that the antibodies will bind to their specific target, a larger surface binding area is required [9].

Zhang *et al.* developed a three-dimensional Y-shaped lattice that increased the surface area, allowing better antigen binding. The isolation interface was built using graphene oxide, polydopamine and streptococcal protein G that interacts with the Fc portion of the antibodies used for exosomal selection [29].

Size-based microfluidic separation separate exosomes from other cellular and molecular components based on their characteristic size (30-150 nm). Exosome Total Isolation Chip (ExoTIC) utilizes a series of nano-filters with decreasing pore size so that particles smaller than exosomes are filtered out, leading to a concentrated exosome suspension. ExoTIC effectively separates exosomes (4-1000 times better than ultracentrifugation) from cell culture media as well as some bodily fluids (urine, bronchoalveolar lavage) [30].

Wang *et al.* created a chip architecture consisting of ciliated micropillars (micropillars with silicone nanowires). The space between the silicone nanowires is equal to the exosome diameter, allowing exosome entrapment between the nanowires and passage of free proteins and nucleic acids. Micropillars provide additional selection by bouncing off apoptotic bodies or larger microvesicles [31] (Figure 3).

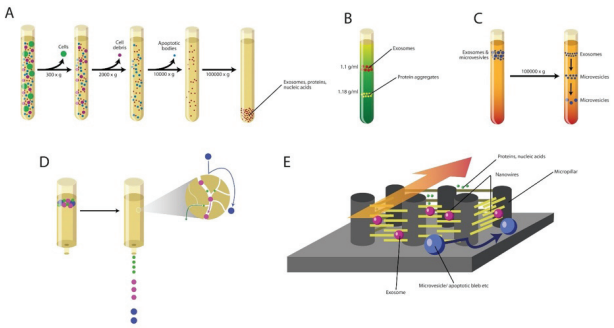

Fig. 3. Exosome isolation techniques. A. Ultracentrifugation involves increasing the centrifugation speed up to 100,000-150,000 x g, when exosomes can be extracted. The exosome suspension is not pure because it is contaminated with nucleic acids and protein aggregates. B. Gradient-density ultracentrifugation uses a density gradient of sucrose/ iodixanol. Based on this density gradient, suspended particles will migrate to their isopycnic point, allowing further separation. C. Moving zone density gradient ultracentrifugation allows microvesicle separation based on their size. D. Size-exclusion chromatography principle. Particles that need to be isolated are suspended in a porous stationary phase. Larger particles (blue) cannot pass between the pores and they travel the fastest through the chromatography column. Small particles (green) enter in the porous material and travel through its microarchitecture, subsequently delaying their elution time. E. Nanowire-based microfluidic separation system. Micropillars provide support for the silicone nanowires and allow size exclusion for the larger particles (blue). Exosomes (magenta) are trapped in the nanowire network because the spacing between the wires is equal to the exosomal diameter. Proteins or nucleic acids (green) pass through the nanowire mesh easily and are rapidly eluted.

CLINICAL APPLICATIONS OF EXOSOMES

Exosomes as diagnostic tools

Exosomal protein and nucleic acid cargo strongly resemble the intracytoplasmic components of the origin cell, offering valuable information in the form of biomolecules [6,32].

Exosomes can be isolated from any extracellular fluid compartment, including blood, urine, pleural fluid, bronchoalveolar lavage, cerebrospinal fluid etc. [1,3], providing a promising, non-invasive alternative to biopsy. Exosomes reflect molecular changes that appear in the parental cells and may function as biomarkers [1]. Moreover, extracellular vesicles' number and cargo are cell-line specific and cell-status specific (physiological or pathological) [10].

Acute myeloid leukemia relapse originates from minimal residual disease and correlates with a poor prognosis. Therefore, early diagnosis and prevention of MRD is one of the main objectives for improving the morbidity and providing longer survival rates. Novel molecules with potential diagnostic role in MRD include the exosomal miR-150, miR-155 and miR-1246. MiR-1246 was also found in the tumor microenvironment as a T-regs-recruiting molecule [6].

In digestive tract pathology, miR-19b-3p and miR-106a isolated from serum exosomes were predictive for gastric cancer and miR-7641 correlated with colorectal cancer evolution.

CD63+ exosomes isolated from urine of diabetic patients was increased at the early stage of renal injury in diabetic nephropathy [10].

Rats subjected to ischemia/reperfusion injury model of acute kidney injury expressed a 60-fold increase of urine exosomal levels containing miR-192 [8].

Exosomes with high EpCAM expression were found in patients with early stage ovarian cancer compared to the control group.

Finally, lung adenocarcinoma-specific exosomes found in plasma were increased in patients with advanced disease compared to healthy volunteers [1].

A new hope: exosomes as drug delivery vehicles

The biochemical composition of exosomes makes them a suitable candidate for drug delivery. The vesicular lipid bilayer surrounds a hydrophilic core, an ideal medium for water-soluble drug transport [7].

Some drugs that in theory have proven to have therapeutic activity might not work in vivo due to their poor pharmacokinetics. One good example is doxorubicin which is coated in liposomal particles to improve its anti-tumoral efficacy and stability in the extracellular milieu. Exosomes, however, provide a better stability in the extracellular space, a longer half-life (implying a larger interval between administrations) and a lower immunogenicity [3].

Due to their small diameter (30-150 nm) [10], exosomes can cross the blood-brain barrier, making them a suitable candidate for drug delivery inside the central nervous system [6,12]. Thomi

et al. demonstrated using Wistar rat pups that infrared labelled MSC (mesenchymal stem cell) derived exosomes administered intranasally prior to an ischemic challenge were uptaken in the central nervous system starting from the frontal lobe (in 30 minutes) and were distributed in the entire brain mass in approximately 3 hours. MSC exosomes significantly reduced neuronal and oligodendrocyte necrosis [33].

The exosomal surface protein repertoire provides additional benefits including low immunogenicity (by FasL and galectin 9 expression [10]), escape from phagocytosis (due to high CD47 expression) and higher tissue- or organ-specificity [7].

Extracellular vesicles loaded with miRNAs or siRNAs can suppress specific gene expression. Supporting evidence proved that MSC-derived exosomes loaded with siRNA specific for the KRAS gene, one of the most prominent genes involved in pancreatic adenocarcinoma, reduced tumor size in a mice model [3].

Zhu *et al.* found that NK-derived exosomes induce apoptosis of B16F10 melanoma cells in vitro and inhibited tumor growth in C57BL/6 mice [34].

Dendritic cell-derived exosomes express HLA class I and class II molecules loaded with peptides facilitating immune priming and effector T and NK cell activation [7]. Therefore, DC-derived exosomes from convalescent patients can provide a novel alternative in vaccine development [1,7,9]. In addition, phase I clinical studies in melanoma patients showed that dendritic cell-derived exosomes have proven to be safe, non-allergic and associated with long-term stabilization and tumor regression by increasing the circulating NK cells and NKG2D (NK group 2 member D)-dependent functions [1].

Drug loading techniques

Drugs can be loaded into exosomal particles either by inducing changes in the parental cells or by modifying the extracellular vesicles after their isolation.

Pre-loading strategies involve drug delivery to the parental cell and subsequently, the drug will be contained within the exosomes at the moment of their release. Techniques include viral transfection and liposomal drug delivery. One of the major issues regarding these methods is that the drug or virus can influence cell metabolism and signaling, leading to an unexpected surface protein signature.

"Surface display" implies cellular transfection with a vector that encodes a chimeric protein formed by an ubiquitous exosomal membrane protein (LAMP2B, lactadherin etc.) fused with the desired peptide sequence that will be expressed on the surface [3].

Post-loading methods imply that the drug is loaded after the exosomes were isolated. In order to effectively load the exosomes, one must assure that the exosomal isolation was done with a high yield and without biological function loss. Techniques include electroporation, sonication and freezing-thawing.

Electroporation is a technique in which an electric current in applied to a cell suspension, resulting in cell membrane pore formation that allows internalization of drugs or nucleic acids inside the cell. Wang *et al.* loaded A549 non-small cell lung cancer-derived exosomes with docetaxel using electroporation. The drug delivering exosomes were administered to a mice model of lung cancer and had a better outcome than the docetaxel therapy alone [35].

Sonication relies on acoustic cavitation of the microbubbles of exosomal suspensions exposed for a short time to ultrasounds. Cavitation provides a transient membrane pore that allows drug passage inside the cell [36].

CRISPR-cas9 loading

CRISPR-Cas9-loaded exosomes provide a promising alternative to viral vectors. Main advantages include low immunogenicity (the outer membrane is recognized as a self-entity) and high cell line specificity due to the similar surface protein signature to the cell of origin [3].

Kim et al. obtained in a murine model of ovarian cancer a significant decrease in tumor size in mice by loading sgRNA and cas9 plasmid into tumor-derived exosomes [37].

CONCLUSION

Initially considered only a cell disposal mechanism, exosomal secretion has proven to be an effective, highly-specific cell communication strategy. This novel type of cell signaling was found to be involved in physiological processes such as immune priming, placental development or tissue regeneration, but also in pathologies such as cancer, diabetes or atherosclerosis. Exosomal isolation is a growing research field for discovering new separation techniques that preserve their physical properties and biological functions.

Exosomes reflect their cell of origin as well as the cell's functional status. Therefore, proteins or nucleic acids isolated from extracellular vesicles can provide early diagnostic or disease progression biomarkers.

Besides their diagnostic potential, drug-loaded exosomal vesicles provide an organ-specific drug delivery, thereby reducing potential adverse effects.

Due to their multivalent role in regulating, exosomes are indeed valuable players in health and disease.

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ROLUL EXOZOMILOR ÎN PROCESELE FIZIOLOGICE ȘI PATOLOGICE UMANE

REZUMAT

Funcționalitatea organismului uman presupune o interacțiune bine-coordonată între diferitele tipuri celulare. De-a lungul evoluției, organismele multicelulare și-au dezvoltat o varietate de căi de comunicare atât la nivelul micromediului local, precum și la distanță. Pe lânga hormoni și interacțiunile dintre proteine, exozomii asigură o strategie de comunicare alternativă cu înaltă stabilitate și specificitate. Penetrabiliatea crescută la nivelul organelor și țesuturilor se datorează imunogenicității și dimensiunilor reduse.

În acest studiu sunt abordate diferite aspecte legate de biogeneza exozomilor, rolul acestora în coordonarea diferitelor interacțiuni fiziologice și patologice, strategii diverse de izolare a exozomilor, precum și potențialele lor aplicații în diagnosticul precoce și transportul de medicamente.

Cuvinte cheie: exozom, inflamație, metastază, angiogeneză, diferențiere celulară, micromediu tumoral

PHYSICAL AGENTS AS ANTIMICROBIAL FACTORS

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ABSTRACT

Antibiotic resistance to most antibiotics currently used is constantly on the rise, thus posing a serious threat to human health. Control of microorganisms is essential to prevent the transmission of diseases and infections and to prevent unwanted microbial contamination. Therefore, it is necessary to develop new strategies by which pathogenic bacteria could be eliminated. The spread of microorganisms can controlled by using physical and chemical agents. Physical agents include control methods such as high or low temperature, desiccation, radiation, osmotic pressure, filtration. The result of the action of these factors is related to their intensity, the duration of time the bacterial cell was exposed, the environment in which it activates, the cell structure, but also its sensitivity to one or more factors. That is why it is necessary to develop new strategies through which pathogenic bacteria can be easily annihilated, as alternatives to, or associated with, antibiotic treatment.

Key words: physical agents, antimicrobial factors, temperature, desiccation, radiation, osmotic pressure, filtration.

INTRODUCTION

Antibiotic-resistant bacteria not only affect human health, but also threaten the safety of patients in hospitals and communities. However, the emergence of drug-resistant bacteria is inevitable due to evolutionary selection but also as a consequence of the non-discriminatory use of antibiotics. In pathogenic bacteria, antibiotic resistance is constantly increasing compared to most antibiotics currently used, thus posing a serious threat to human health. Growing concerns about multiple drug resistance have alarmed the international scientific community. The World Health Organization (WHO) in its global report on antibiotic resistance published in 2014 stated that: "This serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country ". The problem of antibiotic resistance is further amplified by the horizontal transfer of resistance genes among microbial populations. Consequently, not only is human health threatened, but an environmental crisis can also be caused. For example, the contamination of aquatic ecosystems and water reservoirs with antibiotics causes the emergence of multidrug resistance to an alarming level and directly results in increased infection rates.

Therefore, it is necessary to develop new strategies by which pathogenic bacteria could be eliminated. Control of microorganisms is essential to prevent the transmission of diseases and infections and to prevent unwanted microbial contamination. Microorganisms are controlled by physical and chemical agents. Physical agents include control methods, such as high or low temperature, desiccation, radiation, osmotic pressure, filtration. Control by chemical agents refers to the use of disinfectants, antiseptics, antibiotics and chemotherapeutic antimicrobial chemicals. Bacterial activity is influenced by a wide range of physical, chemical and biological factors. The result of the action of these factors is related to their intensity, the bacterial cell exposure time, the environment in which it activates, the cell structure, but also its sensitivity to one or more factors.

Physical agents that can influence the development of microorganisms are:

1. Temperature

Almost all microbial species have a temperature point called "optimal" , at which they carry out their activity normally, and certain limits below and above it, which when reached hinder their development. Thus, from the point of view of the optimal temperature, the bacteria are classified in the following categories [1]:

• mesophiles - pathogenic or conditioned pathogenic species adapted to an optimal temperature of 37-38˚C, human body temperature, with limits between 25-34˚C / 10-45˚C;

• psychrophiles (cryophiles) - which include microorganisms whose optimal temperature is between 15-20˚C with limits between 0 and 20˚C / 0-35˚C;

• thermophilic - include garbage putrefaction bacteria whose optimum temperature is between 50-53˚C with limits between 50-80˚C (85˚C - bacteria in geysers).

High temperature - going beyond the upper limit is harmful,

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leading to disorganization of metabolic reactions through protein coagulation (the theory of protein denaturation and coagulation) or by the accumulation of toxic products resulting from biochemical processes (the intoxication theory). There are differences between the action mode of dry heat and wet heat [2]. In medical practice, heat is used in the process of sterilizing medical instruments and materials used in laboratory diagnosis. Sterilization is performed by dry heat (hot air, incineration) and wet heat (boiling, autoclaving and pasteurization).

a) It is harder for dry heat to penetrate the bacterial cell wall, unlike moist heat. Dry heat is less efficient - for the Clostridium bacillus the temperature required to destroy is 140˚C at an exposure time between 5-15 mind, and a temperature of 180˚C for the Bacillus anthracis at an exposure time of 60 minutes. Dry heat kills microorganisms through a process of protein oxidation, rather than by coagulation of proteins. Examples of dry heat sterilization include [1, 2]:

• Hot air sterilization - very high temperature dry heat is used: 171 ° C for 1 hour; 160 ° C for 2 hours or more; or 121 ° C for 16 hours or more, depending on volume. In general, they are only used to sterilize glassware, metal tools and other inert materials, such as oils and powders that are not damaged by the excessive temperature.

• Incineration - Incinerators are used to destroy disposable materials or consumables by burning.

b) Wet heat penetrates more easily, due to the softening of the cell wall and the increase of its permeability. Wet heat has a bactericidal effect on vegetative forms exposed at 100˚C for 2-3 'or at 60˚C for 1 hour. Bacterial spores are destroyed by autoclaving for 15 'at 121˚C and at a pressure varying between 1.5 atm - 2 atm. Autoclaving, therefore, uses steam under pressure. Normally, water boils at 100 ° C, but when put under pressure, the boiling temperature rises to 121 ° C, enough to destroy the bacterial endospores. The same autoclaving process is used to destroy hepatitis viruses that can survive exposure to boiling water for up to 30 minutes, and the endospores of certain species of Clostridium and Bacillus can survive even hours of boiling [3].

c) Pasteurization is the gentle heating of milk and other materials to destroy certain microorganisms or pathogens. However, it does not kill all microorganisms. The milk is usually pasteurized by flash heating to 71.6 ° C for at least 15 seconds, or 62.9 ° C for 30 minutes by the holding method.

Low temperature - generally inhibits microbial growth by slowing down the microbial metabolism, making bacteria kept at low temperatures no longer multiply and enter latent life [4]. Exceptions to this rule are the meningococcus and gonococcus. More intense cold, below minus 5-10˚C, preserves the biological properties more efficiently than a temperature of 0˚C or - 10˚C. Slow freezing damages bacteria to a greater extent than a sudden freeze, because in the first case ice crystals form, which break the cellular structures and produce saline hyperconcentration with distortion of the colloidal state of proteins. Repeated freezing and thawing is harmful to microbes. Cold finds its applications in the preservation of food, some drugs and laboratory microbial strains (reference bacterial cultures).

Numerous studies have aimed to track the effect of temperature variations on microbial cultures. Thus, Cebrian et al. [5] studied the influence of cultivation temperature on E. coli resistance to 3 different agents: heat, pulsed electric field and hydrogen peroxide. E. coli was grown in a stationary phase at 10 ° C, 20 ° C, 30 ° C, 37 ° C and 42 ° C. The obtained results showed an influence of the cultivation temperature depending on the type of stress applied. Cells subjected to a high culture temperature were also resistant to heat, but were more sensitive to the pulsed electrical fields and hydrogen peroxide, and cells that were cultured at 10 ° C and 20 ° C were much more resistant to pulsed electrical field and hydrogen peroxide.

The results obtained during this investigation support the opinion that the three agents studied act through different mechanisms, due to the different inactivation kinetics observed. Despite the differences between the action mode of the three agents studied, they all involve the membrane at some point, which is an essential structure in bacterial survival under environmental stress. Thus, it seems that cells with more fluid membranes, i.e. those cultured at 10 ° C, would be more sensitive to the action of heat, but more resistant to the formation of pores in the pulse electrical field and the penetration of hydrogen peroxide into cytoplasm.

The study will have to continue in order to clarify the action mechanisms of each agent, and in addition the role of other cellular structures that could be involved in the resistance and the destruction of the cell must be considered. In conclusion, the more heat-tolerant cells were not simultaneously resistant to oxidative stress and pulsed electrical field treatments.

2. Drying (desiccation)

Drying or desiccation in general has a static effect on microorganisms because the lack of water inhibits the action of microbial enzymes. Dehydrated and freeze-dried foods, for example, do not require refrigeration because the absence of water inhibits microbial growth. The sensitivity of bacteria to dehydration differs in relation to the species and even the strain. Microorganisms such as meningococci, gonococci, treponemes, leptospires are very sensitive. Tuberculosis bacilli, staphylococci remain viable for long periods of time. Low temperature, protein environment and the lack of oxygen considerably protect microbes from the harmful effects of dehydration. Lyophilization (cryodesiccation) is based on these principles. The method consists in freezing the microbial suspension in a protective medium, drying it in vacuo and keeping it in closed ampoules with inert gas. Vaccines and reference microbial strains are preserved by lyophilization.

3. Radiation

Radiation acts on bacteria through various mechanisms: the production of bactericidal effect peroxides in the environment (ionizing radiation: X, α, β, γ) and by direct effect on cellular nucleic acids (solar radiation, ultraviolet).

Ionizing radiation (X, α, β, γ, neutrons) have strong bactericidal action, being carriers of higher energies. They cause denaturation of proteins and cellular nucleic acids by inactivation of ribosomes, rupture of nucleic acid chains, inactivation of enzymes due to

the formation of free radicals (OH, H) in the environment, the formation of peroxides (R-O-O-R) that act as oxidizing agents. In small doses, they can produce bacterial mutations (X-rays) with morphological and pathogenicity changes. They're used for sterilization of medical instruments, medicine, food.

Ultraviolet radiation

The ultraviolet portion of the light spectrum includes all radiation with wavelengths from 100 nm to 400 nm. It has a short wavelength and low energy. The bactericidal activity of ultraviolet (UV) light depends on the duration of exposure (the longer the exposure, the longer the bactericidal activity) and the wavelength of the UV rays used (range 260 nm - 270 nm is strongly bactericidal because it acts by selective absorption at the level of nucleic acids) [2].

The mechanism by which UV radiation destroys bacteria: UV light is absorbed by microbial DNA and causes the adjacent thymine bases on the same DNA strand to covalently bind to each other, forming what is called thymine-thymine dimers. As DNA replicates, the nucleotides are not complementary base pairs to the thymine dimers and this ends the replication of that DNA strand.

Most of the damage caused by UV radiation comes from the fact that the cell tries to repair DNA damage through the process called "SOS repair". In very strong DNA that contains a large number of thymine dimers, the SOS repair process is activated as a last effort to bring the DNA back to normal. A DNA that contains many incorrectly embedded bases is synthesized. In other words, UV radiation cause mutations and can lead to poor protein synthesis. With sufficient mutation, the bacterial metabolism is blocked and the organism dies. Agents such as UV radiation that cause high mutation rates are called mutagenic agents. Radiation is used to sterilize rooms, boxes, plastics, medicines.

Among the studies that used radiation to increase antibacterial efficiency, is the one conducted by Chen et al in 2020. They used 2 strains of multi-resistant E. coli that were isolated from urine samples collected in the clinical laboratory, and tested these strains for antibiotic susceptibility. The first strain, called MDR-1, was resistant to penicillin (ampicillin - AMP), tetracycline (tetracycline - TETRA), quinoline (nalidixic acid (nalixide)) and aminoglycosides (streptomycin - STR), and the second isolated strain was also called MDR-2. it is resistant to penicillin (ampicillin - AMP), tetracycline (tetracycline - TETRA), quinoline (nalidixic acid (nalixide)) but also to other aminoglycosides (spectinomycin, gentamicin), chloramphenicol. Bacterial culture of E. coli MDR-2 left overnight was resuspended in fresh LB broth with a final concentration of 10-7-10-8 CFU ml-1. A volume of 10% nanocomposites (abbreviated graphene oxide GO, silver supported on abbreviated graphene oxide GO-Ag) or water (in case of control) was added to the LB broth so that the final concentration of the nanocomposites GO, GO-Ag is 3 and 7μg ml-1, respectively. Before being subjected to laser beam irradiation (808 nm wavelength), the broth was stirred for one hour at 37 ° C. During the irradiation period, the temperature was measured with a thermal chamber, the irradiation time being 7 minutes.

The treated mixed sample was cultured for 6 hours before the bacterial viability was assessed. Among the many uses of the GO-Ag nanocomposite, the authors reported its use for the first time in combination with photo-thermal therapy. The results showed an increase in antibacterial efficiency due to silver nanoparticles that inhibit bacterial growth while graphene oxide sheets absorb NIR light (near-infrared spectroscopy) and generate heat. The conclusion of this study is a promising one in terms of clinical utility. Compared to the widely used AgNP, the GO-Ag nanocomposite showed a much better antibacterial efficiency in clinically isolated multidrug-resistant E. coli. Moreover, photothermal treatment could be combined to lower the antibiotics dose and, in this way, multidrug-resistant E. coli would be completely killed with reduced cytotoxicity. Immunofluorescence images taken to visualize the results showed that the integrity of the bacteria was compromised after treatment with GO-Ag. Given the excellent antibacterial performance of GO-Ag nanocomposites against widespread multidrug-resistant E. coli bacteria, they could be used in conjunction with more common antibacterials in future medical applications.

The effect of electromagnetic radiation with visible range on E. coli samples was studied by Azeemi et al. (2017) in order to find the right dose that could be used in the treatment of diseases caused by E. coli [6]. The strain was isolated from urine samples obtained from hospitalized patients with Urinary Tract Infection (UTI). The presence of E. coli in the obtained samples was identified by standard laboratory methods, by performing both morphological characterization and also biochemical tests. A single colony of E. coli was inoculated into 3 ml of lysis broth. From the suspension obtained, 40 μl were transferred to tubes to be subjected to radiation of visible spectrum of different wavelengths for 18 hours, after irradiation the samples were kept in a stirrer incubator at a temperature of 37 ° C.

Statistical analysis was performed using an IBM SPSS, version 20. A Pearson correlation coefficient was used to evaluate the relation between the optical densities of the irradiated samples and the number of colonies, obtaining a strong positive correlation between the 2 variables. The obtained results showed a different response of the E.coli culture to the 6 waves, the deepest inhibitory effect was at 538 nm characteristic of the green color which proved to be bactericidal in contrast to the wavelength of 453 nm characteristic of the blue color, the sample irradiated at this wavelength recording the largest number of colonies.

Thus, it can be concluded that the radiation in the visible region, i.e. 538 nm (green), 590 nm (yellow) can be effectively used to treat diseases transmitted by E. coli. The effects of visible radiation on E. coli clearly show that different wavelengths affected E. coli differently. All these results indicate that some genetic changes lead to particular morphological behaviors.

The direct effect and determination of the optimal action parameters of LED (blue-red and red-infrared radiation) on the antibiotic sensitivity of the clinical isolates Staphylococcus aureus and the tested strain Staphylococcus aureus ATCC 25923, was studied by Pantyo and collaborators in 2019 [7]. These researchers performed an experiment in which the radiation exposure time of the studied cultures varied from 5 to 25 minutes with an interval of 5 minutes and the radiation frequency was 0 Hz; 10 Hz; 600 Hz; 3000 Hz; 8000 Hz. The research results showed an increase in the sensitivity of the microorganism to some of the antibiotics tested. Based on the data obtained, the researchers involved in the study have developed an algorithm and clinical recommendations for the use of this type of radiation

in complex therapies that are involved in the treatment of inflammatory diseases [7].

The conclusion of this study is that the degree of influence of the applied radiation depends on the wavelength, duration and frequency of radiation pulses - the most pronounced increase in sensitivity was observed at exposure of 5 minutes at 0 Hz (continuous radiation).

Abdulameer and Maki analyzed the effect of photodynamic inactivation on the methicillin-resistant Staphylococcus aureus strain using 2 different lasers: the so-called DPSSL (solid-state laser pumping diodes) at a wavelength of 532 nm combined with a dye (Safranin O) and Laser Diode with a wavelength of 650 nm combined with methyl blue [8]. 100 samples from patients with burns and infected wounds hospitalized in 2 hospitals in Baghdad were tested for antimicrobial susceptibility using the Kirby-Bauer method. The samples were divided into 4 categories: a control category, a category focused on the photosensitization reaction, a category subject to irradiation and a category in which the photosensitization reaction was combined with the irradiation of the samples. The results obtained by the researchers demonstrated the efficiency of the DPSSL laser, registering a small number of cells. Regarding the exposure time, it took 3 minutes in the case of the DPSSL laser compared to 11 minutes in the case of using the Diode Laser, the conclusion of the researchers being that the use of photo-dynamic inactivation as an alternative method in treating infected wounds and superficial burns is viable.

Researchers led by Chaurasia A (2016) propose, following the results obtained, a new treatment based on the action of magnetic nanoparticles against pathogenic bacteria, which block the formation of biofilm and kill bacteria [9]. In this approach, the antibiotic-resistant Staphylococcus aureus strain (USA300) and the uropathogenic Escherichia coli CFT073 are "trapped" in the positively charged magnetic nanoparticles (MCSNP) by electrostatic interaction leading to their complete destruction within 30 minutes, due to the loss of membrane potential when the sample is exposed to a radiofrequency current.

The obtained results demonstrate the efficiency of the method, which can be used as an alternative antibacterial strategy without leading to antibacterial resistance, and can be used successfully in therapeutic applications. In this experiment, Escherichia coli strains (E. coli DH5α and E. coli CFT073, UPEC) were cultured in Luria Bertani broth medium (LB) or LB-agar plates (1.5% agar) at 37 ° C. . Broth cultures were grown under orbital shaking at 200 rpm, while the strains on the plates were grown in an incubator at 37 ° C overnight. Methicillin-resistant Staphylococcus aureus (MRSA) strains were cultured in tryptic soy broth (TSB) or brain-heart infusion medium (BHI) under orbital shaking at 200 rpm or on tryptic soy agar plates (TSA, 1.5% agar) at 37 ° C. Cell growth was monitored by measuring optical density at 600 nm (OD600). Appropriate amounts of antibiotics such as ampicillin (Amp, 100 μg / mL), kanamycin (Km, 50 μg / mL), chloramphenicol (Cm, 33 μg / mL) or streptomycin (Sm, 50 μg / mL) were added to the recombinant E. coli cultures. For TSB cultures and TSA plates, 12.5 and 25 μg / ml chloramphenicol, respectively, were used to culture the recombinant strain S. aureus USA300 FPR3757 that stably expresses the luxBADCE (MRSA) genes integrated into the genome. In order to capture the bacteria and subsequently visualize the MCSNP complex bacteria using an external magnetic field, researchers developed a paramagnetic iron oxide core coated with a silicon coating. The reasons for using MCSNP (magnetic core-shell nanoparticles) are the following:

• prevents the agglomeration of the NP iron core;
• acts as a physical barrier to the nano-bio interface to prevent the biological reduction of Fe (III) to Fe (II) using NADPH, thus reducing the generation of ROS by Fenton, Fenton-type reactions and / or Haber-Weiss;
• prevents passive internalization of NP by bacteria by increasing the size (> 40nm);
• provides a homogeneous interaction surface for the negatively charged bacterial cell.

The group led by Chaurasia proposes a model to explain the disturbances of the microbial membrane following the application of treatment with magnetic nanoparticles exposed to radiofrequency (RMT) [9]. The changes that occur lead to the death of the bacterial cell. These results indicate that physical treatment based on MCSNP (magnetic core-shell nanoparticles) can make for an alternative antibacterial strategy without risk of antibiotic resistance, and can be used for several purposes, both in therapeutic and environmental applications.

4. Ultrasound

Ultrasounds are vibration with a higher frequency than sound waves (over 16,000 Hz). They are obtained with the help of piezoelectric crystals. Ultrasound has a strong bactericidal effect, destroying the bacterial cell relatively fast. In fact, one of the most effective methods of disintegrating microbial bodies - the preliminary stage of chemical analysis of bacteria in the laboratory - is the use of ultrasound on the bacterial suspension. The ultrasonic wave, when passing through extra and intracellular water, determines intense movement of the protoplast with the disintegration of cellular structures (breaking of the cell wall). It is used for disinfection and cleaning of dental and surgical instruments, for chemical analyzes determining the separation of cellular enzymes.

A 2019 study in the International Journal of Hyperthermia aimed to investigate the bactericidal effects of high-intensity focused ultrasound (HIFU) on Bacillus Calmette-Guerin (BCG, a substitute for Mycobacterium tuberculosis) [10]. The conclusion was that HIFU has the therapeutic potential to treat BCG-infected tissues in rats and that a combination of mechanical, cavitation and thermal effects most effectively inactivate BCG bacteria through HIFU. The hope of this study was to provide a plausible basis for an effective non-invasive treatment for tuberculosis.

5. Osmotic pressure

Microorganisms, in their natural environment, are constantly facing changes in osmotic pressure. Water tends to flow through semipermeable membranes, such as the cytoplasmic membrane of microorganisms, to the part with a higher concentration of dissolved materials. In other words, water goes from a higher concentration of water to a lower concentration of water. If the concentration of dissolved substances is higher inside the cell than outside, the cell is in a hypotonic environment, which attracts water into the cell, but the rigid cell walls of bacteria and fungi, however, prevent their explosion or plasmoptysis.

When the concentrations of dissolved substances are the same both inside and outside the cell, the cell is said to be in an isotonic environment. Water passes evenly into and out of

the cell. Hypotonic and isotonic environments are not usually harmful to microorganisms. However, if the concentration of dissolved materials is higher outside the cell than inside, then the cell is in a hypertonic environment. Under this condition, water leaves the cell, resulting in cytoplasmic membrane contraction or plasmolysis. Under such conditions, the cell dehydrates and its growth is inhibited. Young vegetative cells are more sensitive than spores [11].

6. Electricity

Electricity has minor effects on bacteria, but can cause changes in the environment (physico-chemical) with bactericidal side effects: rising temperature, release of chlorine from compounds, ozone formation.

7. Filtration

Filtration is performed by passing a fluid through a porous body (filter). Depending on the pore diameter, the filtration can be clarifying or sterilizing. Microbiological membrane filters provide a useful way to sterilize materials such as vaccines, antibiotic solutions, animal sera, enzyme solutions, vitamin solutions and other solutions that may be damaged or distorted by high temperatures or chemicals [12].

Filters contain pores small enough to prevent the passage of microbes, but large enough to allow fluid to pass through the body. The liquid is then collected in a sterile flask.

Filters with pore diameters from 25 nm to 0.45 μm are usually used in this procedure. A membrane with a porosity of 0.22μm retains all bacteria. Filters can also be used to remove microorganisms from water and air for microbiological testing.

CONCLUSION

In conclusion, it is necessary to develop new strategies as alternatives to or associated with antibiotic treatment, through which pathogenic bacteria can be easily annihilated.

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AGENȚII FIZICI CA FACTORI ANTIMICROBIENI

Rezumat
Rezistența la antibiotice este în continuă creștere față de majoritatea antibioticelor utilizate în prezent, astfel, constituind o amenințare gravă pentru sănătatea umană. Controlul microorganismelor este esențial pentru a preveni transmiterea bolilor și infecțiilor și pentru a preveni contaminarea microbiană nedorită. Prin urmare, este necesară dezvoltarea de noi strategii prin care bacteriile patogene ar putea fi eliminate. Răspândirea microorganismelor poate fi controlată prin intermediul agenților fizici și al agenților chimici. Agenții fizici includ metode de control, cum ar fi temperatura ridicată sau scăzută, desicarea, radiațiile, presiunea osmotică, filtrarea. Rezultatul acțiunii acestor factori este legat de intensitatea acestora, de timpul de expunere a celulei bacteriene, mediul în care activează, structura celulei dar și sensibilitatea ei la unul sau mai mulți factori. De aceea se impune dezvoltarea de noi strategii ca alternative ale tratamentului antibiotic sau asociate cu acesta, prin care bacteriile patogene sa poata fi anihilate cu usurinta.
Cuvine cheie: agenții fizici, factori antimicrobieni, temperatura, desicarea, radiațiile, presiunea osmotică, filtrarea.

ASSESSMENT OF HEART RATE CHANGES TO POSTURE AND ORTHOSTATIC TOLERANCE IN A COHORT OF MALE NIGERIAN UNDERGRADUATES

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ABSTRACT

Background: Orthostatic tolerance and Postural Orthostatic Tachycardia Syndrome (POTS) has been shown to be a common disorder among young adults. We suspect that the prevalence of POTS and orthostatic intolerance among Nigeria young population is likely underreported owing to underdiagnosis. This study assessed orthostatic tolerance and the prevalence of postural orthostatic tachycardia syndrome (POTS) in a cohort of male young adult population of a University.

Methods: Thirty undergraduate male students of the Federal University of Technology Akure (FUTA) aged 15 to 35 years (mean, 21.83 ± 2.82 years) were recruited for the study. The height and weight were measured using standard procedures. The blood pressure and heart rate were evaluated during supine and standing position using a digital sphygmomanometer. The postural heart rate difference (PHRD) was evaluated using established protocol.

Results: The mean ± SD of age, height, weight, BMI, BSA of the participants were 21.83 ± 2.82 years, 1.71 ± 0.06 m, 61.72 ± 7.22 kg, 21.1 ± 1.83 kg/m^2, 1.79 ± 0.12 m^2 respectively. The Supine Heart Rate (HRsup), Standing Heart Rate (HRsta), and Postural Heart Rate Difference (PHRD) were 65.27 ± 9.44, 83.90 ± 13.09, and 18.63 ± 9.64 bpm respectively. Further, the Pulse rate (PR), Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Mean Arterial Pressure (MAP), Pulse Pressure (PP) and Rate Pulse Pressure (RPP) of the participants were 70.77 ± 10.25 bpm; 115.2 ± 9.62; 74.40 ± 9.09; 88.00 ± 8.17; 40.8 ± 9.27 mmHg respectively. Orthostatic intolerance and POTS were observed in 13% of the participants.

Conclusion: These data suggest that orthostatic intolerance with POTS could emerge as a prevalent disorder with health consequences within the Nigerian young population.

Keywords: Tachycardia, Posture, Pressure, Orthostatic Tolerance, Sympathetic, Autonomic, Physiology, POTS

INTRODUCTION

Postural orthostatic tachycardia syndrome (POTS) is an autonomic dysregulation categorized as a disproportionate tachycardia upon standing in the manifestation of orthostatic intolerance [1, 2, 3]. Present adult diagnostic criterion involves a heart rate rise of greater than or equal to 30 bpm within the early 10 minutes of standing or head-up tilt (HUT) in the absence of orthostatic hypotension [4, 5]. Postural orthostatic tachycardia syndrome primarily affects premenopausal females between 15 and 50 years of age, expressing with symptoms of fatigue, headache, sleep disturbance, palpitations, nausea, bloating, and so on [2, 6, 3].

Several pathophysiologic mechanisms; including but not limited to disproportionate sympatho-excitation, autoimmune dysfunction, volume depletion, cardiac and physical deconditioning point to a heterogeneously complex etiology [3, 5, 7]. Most considerably, the devastating nature of postural orthostatic tachycardia syndrome predisposes individuals to a high degree of physiologic dysfunctions and reduced value of life, necessitating more population studies into this classical physiological event of clinical importance [8].

The underlying pathophysiology of postural orthostatic tachycardia syndrome is heterogeneous, encompassing excess sympathetic tone, impaired peripheral autonomic function, cardiovascular deconditioning, volume dysregulation, and autoimmune dysfunction [9]. In apparently healthy subjects, a shift of the intravascular capacity to the interstitial space decreases the overall effective circulating blood volume, showing the gravity-dependent physiology seen in orthostatic-related pathologies.

As expected, a succeeding decrease in stroke volume results in a compensatory increased sympathetic drive augmenting cardiac contractility, heart rate, and systemic vascular resistance. Postural orthostatic tachycardia syndrome patients display persistent reduced stroke volume despite an extravagant sympathetic response with postural changes such as standing, resulting in a final common pathway of tachycardia in the existence of orthostatic intolerance on standing [4, 7].

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METHODS

Participants

This was a cross-sectional descriptive study. The target population was apparently healthy undergraduate students within the age limit of 15 years and 35 years in The Federal University of Technology, Akure. Inclusion criteria were; male, apparently healthy, undergraduate student, Body mass index < 30. Exclusion criteria were; female, suffering from any chronic cardiac disease, less than 15 years of age, greater than 35 years of age, hypertensive, smoker, chronic alcohol taker. A total of 30 volunteers (only males) selected by purposive sampling technique participated in the study.

Anthropometric Measurements

The height (m) and weight (kg) were measured using a stadiometer (Liam Medical England, Model: NO. RGZ-160) coupled with a weighing scale. The body mass index (BMI) and body surface area (BSA) was determined as described elsewhere [17, 18] calculated as follows:

BMI = weight (kg) / [height (m)]^2 [10].

BSA = [11].

Blood Pressure and its derivatives

The systolic blood pressure (mmHg), diastolic blood pressure (mmHg) and pulse rate (beat per minute) were measured using the digital sphygmomanometer (Omron M2 Eco Automatic Upper Arm Blood Pressure Monitor). The inflatable cuff was tied around the left arm of the subjects and the sphygmomanometer recorded the systolic blood pressure, diastolic blood pressure and pulse rate. The systolic blood pressure, diastolic blood pressure and pulse rate were determined at supine position, sitting position and standing position. The mean arterial pressure (MAP), pulse pressure (PP) and rate pressure product (RPP) were determined as follows:

MAP = [(2 x DBP) + SBP] / 3 [12]

Where, MAP is Mean Arterial Pressure in mmHg, DBP is Diastolic Blood Pressure in mmHg and SBP is Systolic Blood Pressure in mmHg.

PP = SBP – DBP [13]

Where, PP is pulse pressure in mmHg, DBP is diastolic blood pressure in mmHg and SBP is systolic blood pressure in mmHg.

RPP = HR X SBP [14]

Where, RPP is Rate Pressure Product in mmHg per minute, HR is the heart rate in beat per minute and SBP is the systolic blood pressure in beat per minute.

Heart rate

The heart rate of the participants were determined at supine and standing position (within 3 to 5 minutes of standing), using a digital sphygmomanometer.

Postural Heart Rate Difference

Postural Heart Rate Difference (PHRD) is determined as the Standing Heart Rate minus the Supine Heart Rate.

PHRD = HRsta - HRsup

Statistical Analysis

All data collected were entered into Microsoft Excel 2013 and imported into SPSS'17 statistical software for analysis. Data were processed using descriptive and inferential analysis. The level of confidence was at $p < 0.05$.

RESULTS

Anthropometric and Socio-demographic Characteristics

The study group was made up of 30 students (all males). The mean age was 21.83 ± 2.82, with the minimum age of 16 and maximum age of 30. The mean height was 1.709 ± 0.06 meters, with the minimum height of 1.56 meters and maximum height of 1.87 meters. The mean weight was 61.72 ± 7.22 kg, with the minimum weight of 49 kg and maximum weight of 80.5. The mean Body Mass Index (BMI) was 21.1 ± 1.83, with the minimum BMI of 17.83 and maximum BMI of 25.99. The mean Body Surface Area (BSA) was 1.79 ± 0.12, with the minimum BSA of 1.46 and maximum of 1.98. All the participants were students of The Federal University of Technology, Akure. The descriptive data is presented in table 1 below.

Heart Rate Response to Posture

The mean heart rate during supine and standing positions were: 65.267 ± 9.436 and 83.90 ± 13.092 respectively. The mean postural heart rate differences (PHRD) was 18.63 ± 9.64. Orthostatic intolerance and Postural Orthostatic Tachycardia Syndrome (POTS) was observed in 13.3% of the subjects. The criteria for POTS was increase in heart rate with greater than or equal to 30 beat per minute within 3 minutes of standing from supine position. The descriptive data of heart rate response to posture is presented in table 3 below while the prevalence of postural orthostatic tachycardia syndrome is represented in figure1.

Table I: Descriptive Statistics of Anthropometric and Socio-Demographic Characteristics

S/n	Variables	Mean ± SD	Minimum	Maximum
1.	Age (Years)	21.83 ± 2.82	16	30
2.	Height (m)	1.71 ± 0.06	1.56	1.87
3.	Weight (kg)	61.72 ± 7.22	49	80.5
4.	BMI (kg/m^2)	21.1 ± 1.83	17.83	25.99
5.	BSA (m^2)	1.79 ± 0.12	1.46	1.98

Table II: Descriptive Statistics of Blood Pressure Parameters and Its Derivatives

S/n	Variables	Mean ± SD	Minimum	Maximum
1.	SBP (mmHg)	115.2 ± 9.62	98	133
2.	DBP (mmHg)	74.40 ± 9.09	50	88
3.	PR (bpm)	70.77 ± 10.25	52	92
4.	MAP (mmHg)	88.00 ± 8.17	67.33	102.33
5.	PP (mmHg)	40.8 ± 9.27	26	60
6.	RPP (mmHg)	8173.17 ± 1519.70	5400	12144

Table III: Descriptive Statistics of Heart Rate Response to Posture

S/n	Variables	Mean ± SD	Minimum	Maximum
1.	Supine Heart Rate (bpm)	65.27 ± 9.44	48	92
2.	Standing Heart Rate (bpm)	83.90 ± 13.09	63	112
3.	PHRD (bpm)	18.63 ± 9.64	1	47

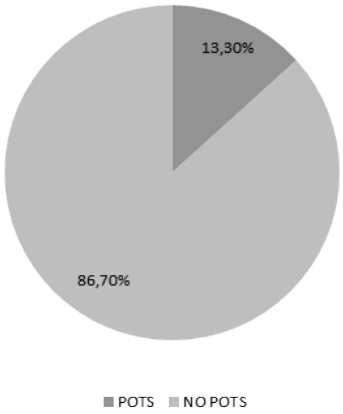

Fig. 1: Prevalence of postural orthostatic tachycardia syndrome (POTS).

DISCUSSION

The result shows 13.30 % prevalence of postural orthostatic tachycardia syndrome (POTS) among undergraduate students at The Federal University of Technology, Akure. POTS is a disorder in which the autonomic nervous system fails to compensate for the upright position of the body upon standing from supine position [15]. When lying in supine position, up to 30% of the blood volume is occupied in the thorax. During standing, 300–800 mL of blood is pulled downwards from the thorax into the abdomen and lower extremities due to force of gravity. Most of this pooling into lower extremities veins occurs within 10 seconds of standing. This results in a decrease in venous return to the right side of the heart with a subsequent decrease in the stroke volume and cardiac output. Reductions in pulse pressure and stroke volume are detected by arterial baroreceptors (located in carotid sinuses and the aortic arch) and cardiopulmonary mechanoreceptors (located in heart and lung). Compensatory reflexes results in elevated sympathetic nervous system output (peripheral arteriolar vasoconstriction) and depressed parasympathetic nervous system output (reduced vagal tone to the heart with cardio-acceleration) [16]. After standing in normal subjects, there is a 10–15 bpm increase in heart rate, but in people with POTS, the increase in heart rate is exaggerated to about 30 beats per minutes or more. Elevated heart rate in POTS patients is due to exaggerated sympathetic nervous system which can be attributed to any of the following: autonomic dysfunctions, hyperadrenergic response to standing, genetic composition of individuals, hypovolemia, and impaired cerebral autoregulation [16]

CONCLUSION

These data suggest that orthostatic intolerance with POTS could emerge as a prevalent disorder with health consequences within the Nigerian young population. Hence more studies are warranted to determine the prevalence within the larger population and geographical locations.

ETHICAL CONSIDERATIONS

Ethical standards regarding studies on human subject were strictly followed. Informed consent of each participant before and throughout the study were obtained in accordance with standard practice concerning research relating to human subjects.

CONFLICTS OF INTEREST

Authors declare that there are no conflicts of interest concerning this publication whatsoever.

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EVALUAREA MODIFICĂRILOR RITMULUI CARDIAC LA POSTURA ȘI TOLERANȚA ORTOSTATICĂ LA O COHORTĂ DE STUDENȚI NIGERIENI DE SEX MASCULIN

REZUMAT

Toleranța ortostatică **și** sindromul tahicardiei ortostatice posturale (POTS) reprezintă o tulburare frecventă la adulții tineri. Se presupune că prevalența POTS **și** a intoleranței ortostatice **în** rândul populației tinere din Nigeria este probabil subdeclarată din cauza subdiagnosticului. Acest studiu a evaluat toleranța ortostatică **și** prevalența sindromului de tahicardie ortostatică posturală (POTS) **într**-o cohortă de populație adultă tânără de sex masculin dintr-o universitate.

Metode: 30 de studenți de la Universitatea Federală de Tehnologie Akure (FUTA) cu vârste cuprinse **între** 15 **și** 35 de ani (medie, 21,83 ± 2,82 ani) au fost recrutați pentru studiu. **Înălțimea și** greutatea au fost măsurate utilizând proceduri standard. Tensiunea arterială **și** frecvența cardiacă au fost evaluate **în** decubit dorsal **și în** ortostatism, utilizând un sfigmomanometru digital. Diferența de ritm cardiac postural (PHRD) a fost evaluată utilizând protocolul stabilit.

Rezultate: Media ± SD a vârstei, **înălțimii**, greutății, IMC, BSA a participanților a fost de 21,83 ± 2,82 ani, 1,71 ± 0,06 m, 61,72 ± 7,22 kg, 21,1 ± 1,83 kg / m2, 1,79 ± 0,12 m2, respectiv. Ritmul cardiac **în** decubit (HRsup), ritmul cardiac **în** picioare (HRsta) **și** diferența de frecvență cardiacă posturală (PHRD) au fost de 65,27 ± 9,44, 83,90 ± 13,09 **și** respectiv 18,63 ± 9,64 bpm. Mai mult, rata pulsului (PR), tensiunea arterială sistolică (SBP), tensiunea arterială diastolică (DBP), presiunea arterială medie (MAP), presiunea pulsului (PP) **și** presiunea pulsului (RPP) a participanților au fost de 70,77 ± 10,25 bpm; 115,2 ± 9,62; 74,40 ± 9,09; 88,00 ± 8,17; 40,8 ± 9,27 mmHg, respectiv. Intoleranța ortostatică **și** POTS au fost observate la 13% dintre participanți.

Concluzie: Aceste date sugerează că intoleranța ortostatică cu POTS ar putea apărea ca o tulburare prevalentă cu consecințe asupra sănătății **în** cadrul populației tinere nigeriene.

Cuvinte cheie: Tahicardie, Postură, Presiune, Toleranță ortostatică, Simpatică, Autonomă, Fiziologie, POTS

EXPLORATION OF THE ASSOCIATION BETWEEN CLASSICAL AND EMERGING RISK FACTORS FOR DIABETIC KIDNEY DISEASE AND ALBUMINURIA IN A COHORT OF TYPE 2 DIABETES MELLITUS PATIENTS

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ABSTRACT
The presence of albuminuria in patients with type 2 diabetes mellitus is a marker of endothelial dysfunction and also one of the criteria for diagnosing diabetic kidney disease. The present study aimed to identify associations between cardiovascular risk factors and renal albumin excretion in a group of 218 patients with type 2 diabetes mellitus. HbA1c values, systolic blood pressure, diastolic blood pressure were statistically significantly higher in patients with microalbuinuria or macroalbuminuria compared to patients with normoalbuminuria ($p < 0.01$). We identified a statistically significant positive association between uric acid values and albuminuria, respectively 25- (OH)2 vitamin D3 deficiency and microalbuminuria ($p < 0.01$).
Keywords: diabetes, microalbuminuria, hyperuricemia, vitamin D.

1. INTRODUCTION

Patients with diabetes mellitus are about 2 times more likely to suffer from chronic kidney disease than people who do not have diabetes (1). In the NHANES study in patients evaluated from 2009 to 2014, the prevalence of chronic kidney disease in patients with diabetes mellitus was 26.2%, the diagnostic criteria being the presence of albuminuria (2). In diabetes mellitus patients over 65 years of age, the prevalence of reduced glomerular filtration rate was higher than in young patients, while albuminuria had comparable prevalence in both categories. The prevalence of chronic kidney disease in patients with diabetes mellitus in 2008 in Shanghai, China was 33.5% (3). In Singapore the prevalence of chronic kidney disease in patients with diabetes was 53%, in 21% of patients a glomerular filtration rate <60mL/min/1.73m^2 was identified, 48% had albuminuria ≥30mg/g, and 28% they had diabetic retinopathy (4). In literature the factors that were associated with diabetic kidney disease were the presence of diabetic neuropathy, hypertension, hypertriglyceridemia, increased BMI, increased duration of diabetes, HbA1c≥8% and the presence of cardiovascular disease.

Hyperuricemia is being more frequently recognized as a risk factor for diabetic kidney disease or (5). Numerous studies indicate that hyperuricemia is independently associated with chronic kidney disease (6,7,8,9). According to a cross-sectional study that included 2,108 Chinese patients with type 2 diabetes, there was a positive correlation between uric acid values and albuminuria and impaired renal function (10). Another study demonstrated that during a 5-year follow-up of 1,449 type 2 diabetic patients with normal renal function, the presence of hyperuricemia was identified as an independent risk factor for the development of diabetic kidney disease (11). Although the pathogenesis of diabetic kidney disease has not been fully investigated, it is currently thought to be related to glucose and lipid metabolism disorders, renal hemodynamic abnormalities, oxidative stress, elevated vasoactive substances, cytokines and genetic factors. Numerous studies have identified a clear correlation between vitamin D deficiency and type 2 diabetes, and vitamin D deficiency is quite common in patients with chronic renal failure (12). Therefore, numerous risk factors for diabetic kidney disease are being recognized contributing to a more profound understanding of this disease.

The present study aims to evaluate the correlation between albuminuria in patients with type 2 diabetes and uric acid and the correlation between albuminuria and vitamin D.

2. MATERIAL AND METHOD

The present study included patients with type 2 diabetes who were evaluated at the County Clinical Hospital in Oradea, Bihor. The inclusion criteria applied for this investigation were: age between 18 and 75 years, type 2 diabetes confirmed by HbA1c determination, individuals who agreed to be investigated. The exclusion criteria were: patients with glomerular filtration rate <15 mL/min/1.73m2 or those on dialysis, patients with

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liver cirrhosis, patients with neoplastic diseases, patients with mental illness, patients with hypertension grade III defined as SBP≥180mmHg or DBP≥120mmHg, patients suffering from glomerular or tubular diseases, patients with HbA1c >12%, patients with acute or chronic urinary tract infections. The study was done between September 1, 2019 and March 31, 2020. A number of 299 were initially considered eligible for inclusion in the study but after applying the inclusion and exclusion criteria only 218 of them remained in the study. The research was conducted with the approval of the Ethics Commission of Oradea County Hospital. The glomerular filtration rate was calculated using the CKD-EPI equation. In addition to the usual laboratory determinations like HbA1c, lipid profile, creatinine, urea, it was done the determination of the albumin/creatinine ratio in the urine spot, the serum value of uric acid and the serum value of 1,25-(OH)2-vitamin D3. Turbidimetry was used for determination of albumin/creatinine ratio, determination of 1,25-(OH)2-vitamin D3 was performed by chemiluminescence and uric acid by colorimetric enzymatic method. All the examinations mentioned above were performed in the hospital laboratory. The presence of microalbuminuria or macroalbuminuria was considered a diagnostic criterion for impaired renal function, we did not use the diagnosis of chronic kidney disease because we could not document the presence of a persistent elevated albuminuria. Microalbuminuria was defined as estimated albumin/creatinine ratio on between 30-300mg/g and macroalbuminuria as albumin/creatinine ratio ≥300mg /g. Values of 1,25-(OH)2-vitamin D3 >50 nmol/L were considered normal and vitamin D deficiency was defined as 1,25-(OH)2-vitamin D3 ≤50 nmol/L. Hyperuricemia was defined as the serum value of uric acid ≥7mg/dL. Good control of glycaemic values was defined as HbA1c <7%. A binary variable was defined, the deficiency of 25-(OH)2-vitamin D3, with a value of 1 if 1,25-(OH)2-vitamin D3 ≤50 nmol/L and 0 if 1,25-(OH)2-vitamin D3 >50 nmol/L.

Statistical processing was performed using Biostat software. The average values of the different parameters were compared using the Anova test. A value of p <0.05 was considered statistically significant. Univariate regression and multivariate regression were used to assess correlation between variables.

3. RESULTS AND DISCUSSION

Table 1. Clinical-biochemical characteristics according to the level of albuminuria

Parameter	Normoalbuminuria (n=94)	Microalbuminuria (n=104)	Macroalbuminuria (n=20)	p
Age	48.12 ± 8.71	50.78±8.56	50.25±8.36	0.03
sex (%men)	67.74%	50.96%	65.00%	0.01
Creatinine (mg/dL)	0.74 ± 0.16	0.68±0.16	0.80±0.22	0.098
Urea (mg/dL)	18.38 ± 4.38	17.53±4.08	19.43±5.19	0.15
HbA1c (%)	7.80 ± 1.38	8.35±1.49	8.32±1.28	0.0088
Albumin/creatinine ratio (mg/g)	19.99 ± (15.75,24.85)	80.71 (39.93,90.84)	569.43 (354.57,640.78)	0.0001
BMI (kg/m^2)	26.78 ± 3.91	27.34±3.89	28.95±4.19	0.31
Hypertension (%)	45.16	59.61	90.00	0.0001
SBP (mmHg)	128.55 ± 14.03	135.25±16.49	141.35±15.56	0.0026
DBP (mmHg)	79.23 ± 9.25	83±10.51	87.05±9.01	0.0087
GFR (mL/min/1.73m^2)	105.11 ± 11.48	105.83±10.63	99.01±16.88	0.67
1,25-(OH)2 Vitamin D 3(nmol/L)	45.40 ± 16.16	40.54±13.84	38.92±18.13	0.045
Uric acid (mg/dL)	5.49 ± 1.22	5.55±1.54	6.27±1.53	0.76
Diabetes duration	6.12 ± 4.94	6.38±5.79	8.03±6.17	0.74
Colesterol (mg/dL)	180.85 ± 40.73	186.89±41.21	173.24±41.76	0.68
LDL-cholesterol (mg/dL)	123.40 ± 31.63	121.59±37.18	103.8±26.33	0.77
Tryglicerides (mg/dL)	175.20 ± (94.76,183.33)	201.64 (99.19,232.27)	275.98 (123.99,266.81)	0.26
HDL-cholesterol (mg/dL)	42.59 ± 11.05	43.18±12.44	39.38±12.51	0.72

Correlation between uric acid values and albumin/creatinine ratio

In figure 1 it is shown that there is a statistically significant correlation between the value of serum uric acid and the urinary albumin/creatinine ratio. High uric acid levels are associated with elevated urinary albumin/creatinine ratio (p=0,01).

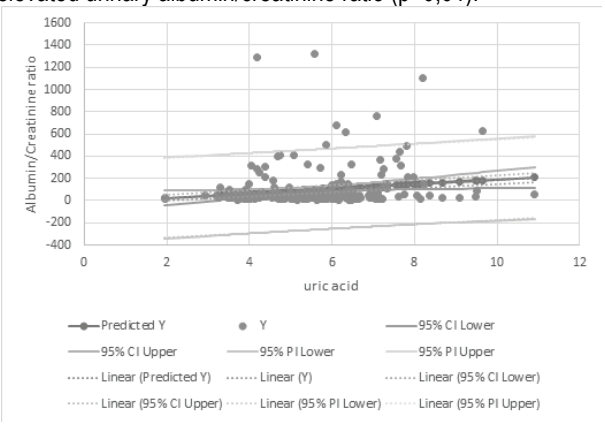

Fig.1. Correlation between serum uric acid value and albumin/creatinine ratio

Correlation between 1,25-(OH)2-vitamin D3 values and albumin/creatinine ratio

In figure 2 it is shown that there is no statistically significant correlation between serum 1,25-(OH)2-vitamin D3 and urinary albumin / creatinine ratio (p=0.13).

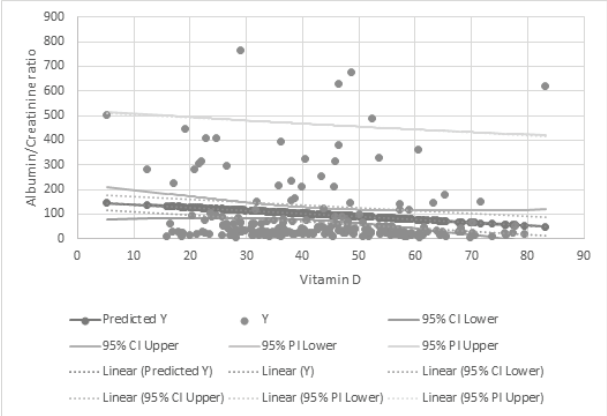

Fig. 2. Correlation between serum 1,25-(OH)2-vitamin D3 value and albumin/creatinine ratio

Table 2. Linear regression parameters between serum uric acid value and albumin/creatinine ratio and linear regression parameters between 1,25-(OH)2-vitamin D3 value and albumin /creatinine ratio

Paramter	*Coefficient*	*Standard error*	*Lower confidence interval*	*Upper confidence interval*	*t Static*	*p-value*
Uric acid	20.7728	8.7136	3.5978	37.9478	2.3840	0.0180
1,25-(OH)2-vitamin D3 value	-1.2347	0.8146	-2.8403	0.3710	-1.5156	0.1311

Correlation between 25-(OH)2-vitamin D3 status and albumin/creatine ratio after adjustment based on multiple factors

A positive association has been found has been found between 25-(OH)2-vitamin D3 deficiency and albumin/creatine ratio.

Table 3. Correlation between 25-(OH)2-vitamin D3 status and albumin/creatine ratio after adjustment for multiple factors by multiple linear regression

Parameter	*Coefficient*	*Standard error*	*Lower confidence interval*	*Upper confidence interval*	*t Static*	*p-value*
Intercept	-1.1631	0.4732	-2.0960	-0.2302	-2.4582	0.0148
Age	0.0057	0.0048	-0.0038	0.0152	1.1790	0.2398
Sex	-0.0583	0.0817	-0.2195	0.1028	-0.7133	0.4765
HbA1c	0.0630	0.0232	0.0173	0.1087	2.7168	0.0072
BMI	0.0030	0.0093	-0.0153	0.0213	0.3253	0.7453
SBP	0.0050	0.0023	0.0005	0.0095	2.1745	0.0308
Uric acid	0.0407	0.0265	-0.0116	0.0930	1.5339	0.1266
Diabetes duration	-0.0015	0.0068	-0.0148	0.0119	-0.2146	0.8303
Cholesterol	-0.0008	0.0046	-0.0100	0.0083	-0.1758	0.8606
LDL-cholesterol	-0.0005	0.0047	-0.0097	0.0087	-0.1138	0.9095
Trygliceri-des	0.0001	0.0006	-0.0010	0.0013	0.2415	0.8094
HDL-cholesterol	0.0022	0.0052	-0.0081	0.0124	0.4158	0.6780
1,25-(OH)2-vitamin D3 deficiency	0.1649	0.0739	0.0193	0.3106	2.2325	0.0267

Our results are in concordance with the results from literature demonstrating that hyperuricemia and vitamin D deficiency play a role in the pathogenesis of diabetic kidney disease. It has been demonstrated that uric acid increases oxidative stress and promotes the activation of the renin-angiotensin-aldosterone system (13, 14). Uric acid is associated with early diabetic kidney disease (15, 16), and can be a factor that can predict the progression of microalbuminuria (17). Observational studies have demonstrated that serum uric acid and microalbuminuria levels were significantly positively correlated with kidney disease in patients with type 2 diabetes (18). Patients with higher uric acid levels have worse renal function, regardless of glycated haemoglobin (HbA1c) or the duration of diabetes mellitus (19). Xanthine oxidase (XO) is a very important enzyme that has the role for converting sulfhydryl groups to uric acid. Data from literature has demonstrated that an increase in uric acid by 1 μmol/L increased the risk of albuminuria by 1.5%, and an increase in XO activity by 1 U/L increased the risk of albuminuria by 1.5%. In diabetes mellitus patients, both XO and uric acid are independently associated with albuminuria (20).

Numerous data support the association between low 1,25-(OH)2-vitamin D3 serum values and worse renal function. In a study done on 1,216 patients with diabetes was identified an independent association between vitamin D deficiency and the onset of diabetic kidney disease (21). Levin and colleagues

demonstrated that in diabetes mellitus patients low serum levels of 1,25-(OH)2-vitamin D3 were independently associated with lower glomerular filtration rate and with a high urinary albumin-creatinine ratio (22). Also, supplementation with vitamin D can be helpful in reducing the mortality of diabetes mellitus patients with very advanced kidney disease, in a study done on haemodialysis patients it was found that patients who received vitamin D supplements had a significantly higher 2-year survival rate than patients who did not receive (23), suggesting efficacy. vitamin D in reducing mortality in patients with end-stage renal disease. Furthermore, animal studies have shown that serum levels of 25 (OH) vitamin D and 1.25 (OH) 2 vitamin D3 were significantly reduced in rats with diabetic kidney disease and treatment with rat vitamin D effectively inhibited proliferation of mesangial cells induced by hyperglycaemia (24). In another animal study, diabetic mice with vitamin D -/- receptor mutations had more severe renal impairment and were more likely to have severe proteinuria and glomerulosclerosis compared with wild-type mice (25).

4. CONCLUSION

In our study we demonstrated a statistically significant correlation between uric acid levels and albumin/creatinine ratio in univariate regression and between 25-(OH)2-vitamin D3 deficiency and albumin/creatinine ration in multivariate regression. The role of hyperuricemia and 1,25-(OH)2 vitamin D3 need to be further explored for a better understanding of the pathogenesis of diabetic kidney disease.

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Conflict of interest statement: none declared.

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EXPLORAREA ASOCIERII ÎNTRE FACTORII DE RISC CUNOSCUȚI ȘI EMERGENȚI PENTRU BOALA RENALA DIABETICĂ ȘI RESPECTIV ALBUMINURIE LA PACIENȚII CU DIABET ZAHARAT DE TIP 2

Rezumat
Prezența albuminuriei la pacienții cu diabet zaharat tip 2 este un marker al disfuncției endoteliale și totodată unul dintre criteriile diagnosticului bolii renale diabetice. Studiul de față a avut ca scop identificarea asocierilor între factorii de risc cardiovascular și excreția renală de albumină la un grup de 218 pacienți cu diabet zaharat de tip 2. Valorile HbA1c, tensiunii arteriale sistolice, tensiunii arteriale diastolice au fost semnificativ statistic mai mari la pacienții cu microalbuinurie sau macroalbuminurie comparativ cu pacienții cu normoalbuminurie ($p<0.01$). Am identificat o asociere pozitiva statistic semnificativă între valorile acidului uric și albuminurie respectiv deficiența de 25-(OH)2 vitamina D3 și microalbuminurie ($p<0.01$).
Cuvinte cheie: diabet zaharat, microalbuminurie, hiperuricemie, vitamina D

NECROTIZING FASCIITIS IN A PATIENT WITH HEPATIC ENCEPHALOPATHY. CASE REPORT AND LITERATURE REVIEW

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ABSTRACT

Introduction: Necrotizing fasciitis, considered to be a very rare and severe soft tissue infection, is associated with rapid progressive necrosis of subcutaneous tissue and superficial fascia. Although this rare condition has a very high mortality rate and requires prompt diagnosis as well as urgent treatment, there are no defined blood tests that can diagnose it. **Case presentation:** In this report, we present the case of a 51-year-old patient, known with mental and behavioral disorders due to alcohol abuse, liver cirrhosis, early liver encephalopathy, anemia, and hypoproteinemia, who presented to the Dermatovenerology department with a main complaint of hematoma at the level of the joint of elbow, appeared as a result of physical aggression. Despite the medical care, the patient's situation deteriorated, more accurately with the extended progression of erythema and sorrow, with deep hyperpigmentation and extended tenderness. As a consequence, the patient was transferred urgently to the plastic surgery department with the suspicion of necrotizing fasciitis as a diagnosis. **Conclusion:** Knowing that soft tissue necrotizing infections have an acute, aggressive, and rapidly progressive character, in patients with cirrhosis they occur more often than in the general population, because the phenomenon of bacterial translocation occurs in the intestinal lumen as a consequence of intestinal bacterial growth, increased permeability and decreased motility. Excessive growth of intestinal bacteria is also responsible for the occurrence of hyperammonemia, leading, consequently, to the appearance of hepatic encephalopathy. Considering those presented, complications place this group of patients in the category of those at high risk of mortality.

Keywords: fasciitis, cirrhosis, encephalopathy, necrosis, bacterial translocation

INTRODUCTION

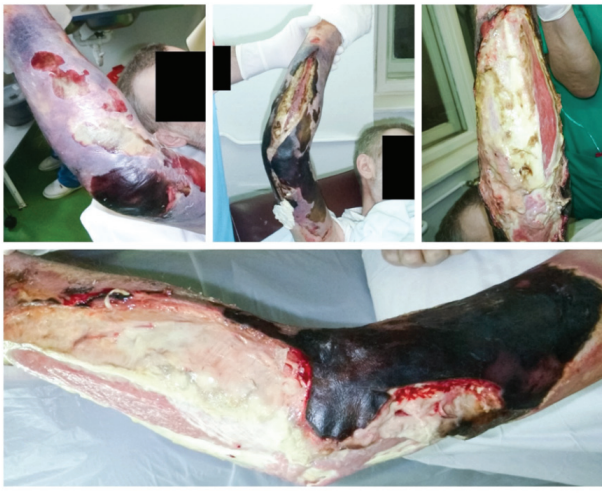
Necrotizing fasciitis is considered to be a very rare and severe soft tissue infection, associated with rapid progressive necrosis of subcutaneous tissue and superficial fascia. Although this rare condition has a very high mortality rate and requires prompt diagnosis as well as urgent treatment, there are no defined blood tests that can diagnose it. At the onset, it is difficult to differentiate from other superficial skin conditions, so early detection and surgical debridement, as well as an aggressive antimicrobial therapy, are essential to limit the loss of limb or life [1]. Clinical features of necrotizing fasciitis include severe regional cellulitis with ill-defined margins, soft-tissue edema with grayish-brown discharge, severe pain, skin necrosis with bullae formation (blisters containing serous fluid), crepitus (grating sound), and sloughing of the skin followed by numbness of the involved region due to destruction of nerves in the fascial planes [17,18]. The bacteria can also result in ischemia of the involved region due to thrombosis of blood vessels which eventually results in gangrene. Limbs are the most common site involved in this life-threatening disease, followed by the perineum and trunk [17,19].

Received 5th of June 2021. Accepted 7h of July 2021. Chiorean Diana Maria, County Clinical Hospital of Târgu Mureș, 6 Bernády György Square, 540072, Târgu Mureș, Mureș, România. Telephone: +40760513978. E-mail: chioreandianamaria@yahoo.com

CASE PRESENTATION

We present the case of a 51-year-old male patient, known with mental and behavioral disorders, hepatic cirrhosis, and early hepatic encephalopathy due to alcohol abuse. He presented to the dermatovenerology department with a main complaint of hematoma at the level of the joint of the elbow, which appeared as a result of physical aggression. At admission, the patient's psychopathological picture was dominated by psychomotor anxiety, extremity tremor, nausea, vomiting, profuse sweating, toxicophilic behavior, overall decreased efficiency, and irritability. Throughout the hospital stay, a diagnostic of erysipelas has been established and the patient was treated with five doses of Metamizole sodium monohydrate 1g/2ml intravenous (i.v.), two doses of Alprazolam 1mg i.v., ten doses of Ceftriaxone 1g i.v., one dose of Dexamethasone 8mg/2ml i.v., ten doses of Gentamicin sulfate 40mg/ml i.v., ten doses of Omeprazole 20mg, 500 ml of lactated Ringer i.v., four doses of Diclofenac sodium 75mg/ml i.v., fifty tubes of Gentamicin sulfate ointment 0,1% and two-hundred tubes of Diclofenac sodium gel 5%/1% menthol. No blood tests or X-Rays were performed. Despite the medical care, the patient's situation deteriorated, more accurately with the extended progression of erythema and sorrow, with deep hyperpigmentation and extended tenderness. As a consequence, the patient was transferred urgently to the plastic surgery department with the suspicion of necrotizing fasciitis as a diagnosis.

On laboratory testing, the metabolic panel was notable for a gamma-GT of 402 U/L, low density lipoprotein 170 mg/dL, potassium 6,14 mmol/L, sodium 133 mmol/L, urea 72 mg/dL. Complete blood count revealed the number of leukocytes as 30,86/µL, neutrophiles 26,18/µL, monocytes 1,30/µL, bazophils 0,44/µL, eritrocytes 3,88/µL, HEM 33,71 pg, VTM 11,18/fL, PCT 0,37%, neutrophiles 84,83%, limphocytes 9,09%.



The biochemistry part was notable for uric acid as 7,93 mg/dL and alanine transaminase as 120 U/L.

Emergency debridement was performed (Figure 1). Under troncular anesthesia, the incision and wide excision of the septic-necrotic tissue were performed, which revealed subcutaneous edema. During the surgical procedure, a purulent material was collected, which revealed a positive response for group A beta-hemolytic streptococcus, penicillin-sensitive. The patient was treated with Amoxicillin/ Clavulanic acid, Fluconazole, Gentamicin, Metronidazole, lactated Ringer, Plasma volume substituent 6%, Alprazolam, Omeprazole. The infection was kept under control due to antibiotics and the patient's status improved. After the patient has been discharged, we had no longer information regarding the evolution of his health condition. We kept him under observation for a period of twenty-one days.

Fig. 1. Images of the emergency debridement

DISCUSSIONS

Even though necrotizing fasciitis has been a known clinical element for a considerable length of time, the term was first utilized by Wilson in 1952 to portray „fascial corruption" [5]. As referenced in the past lines, pathogens can spread quickly along the fascial planes. When the microbes are particularly inoculated, the toxins spread through the skin, from the epidermis to profound muscle tissue in a quickly spreading way, causing corruption of the wide tissue and its destruction [10]. Therefore, the expression „time is fascia" [3] appears to be proper, which has led to the built-up conviction that controlling sources through the early medical procedure [6] to expel necrotic and tainted tissue can diminish the movement of the contamination and improve the results [7]. Complications observed with a higher risk of death, incorporate diabetes, stoutness, congestive cardiovascular breakdown, fringe vascular ailment, constant liver malady, and malignancies [14]. Alluding to our case, contaminations are basic in patients with hepatic cirrhosis. They occur more regularly in these categories of patients than worldwide. One possible reason behind this is the bacterial translocation of the intestinal lumen that occurs due to intestinal bacterial abundance, extended permeability, and decreased motility [2]. The abundance of intestinal bacteria is also responsible for hyperammonemia, causing hepatic encephalopathy [9]. Patients with cirrhosis and necrotizing fasciitis require long-term hospitalization and charge a large number of hospital fees, making necrotizing fasciitis the hospital's most expensive diagnosis [1]. Direct inoculation of adjacent necrotic tissue or spread in the lymphatic tract can lead to the establishment of extremity infection and is therefore considered a fatal skin disease [11]. According to reports, its mortality rate is very high, about 4.8 per 1,000,000 years per person [8]. It can affect all age groups, especially older men, with a total death rate of almost 30%, which will increase at extreme ages. Regarding treatment, one with 100% oxygen at atmospheric pressure of two to three times can improve the oxidative explosion of leukocytes, limit the development of anaerobes and restrict the arrival of bacterial poisons, however, it should not delay the medical crisis, procedure, and strong fluid invigoration [12].

PATHOLOGICAL ASPECTS

Necrotizing fasciitis can progress in two manners:

1. The penetrant manner, where organisms or the spores get inoculated via a break in the integrity of skin or mucosa;
2. The nonpenetrant manner, where a deep tissue injury in individuals with transient bacteremia leads to an influx of organisms that gain access via deep tissue vessels to the surrounding tissues.

In both manners of the entrance, microbes once inoculated, release exotoxins altering host repair mechanisms leading to leucocyte–platelet aggregation resulting in widespread tissue damage, occlusion of vessels causing tissue necrosis, and exaggerated inflammatory responses [4],[13]. Invasion of these microbes is recognized by the host immune system leading to a widespread influx of acute inflammatory cells with an aim to kill these pathogens [4]. Regarding the type of infection, these can be monomicrobial or polymicrobial, with causative agents as anaerobes (Clostridium perfringens, Bacteroides fragilis), Streptococcus species (including group A beta-hemolytic streptococci), and Staphylococcus species. Group A Streptococcus is well established as the most common pathogen causing necrotizing fasciitis [16]. This kind of bacteria can cause a wide variety of infections that range from minor illnesses, such as strep throat and mild skin infection, to very severe and life-threatening post-streptococcal diseases, such as rheumatic fever and glomerulonephritis [17].

From a histopathological point of view, as in the case of our patient, the microscopically evaluated skin fragments were interested in an extensive process of deep necrosis of the epidermis, dermis, and even subcutaneous tissue. A moderate polymorphic inflammatory infiltrate consisting of granulocytes and lymphocytes was identified. Rare plasma cells and rare eosinophils have also been observed (Figure 2).

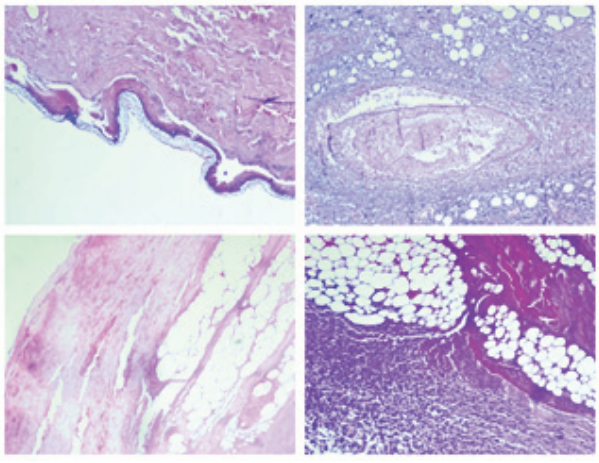


Fig. 2. Images of the microscopic perspective – hematoxilin and eosine, 10 x magnification

CONCLUSION

Our case raises the possibility that cirrhosis could lead to necrotizing fasciitis because of the simultaneous presence of additional predisposing factors. Although a definitive connection cannot be made based on our case alone, we suggest further investigations regarding this aspect.

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FASCEITA NECROTIZANTĂ LA UN PACIENT CU ENCEFALOPATIE HEPATICĂ. PREZENTARE DE CAZ ȘI ANALIZA LITERATURII DE SPECIALITATE

REZUMAT

Introducere: Fasceita necrotizantă, considerată a fi o infecție foarte rară și severă a țesuturilor moi, este asociată cu necroza rapid progresivă a țesutului subcutanat și a fasciei superficiale. Deși această afecțiune rară are o rată de mortalitate foarte ridicată și necesită un diagnostic prompt precum și un tratament urgent, nu există teste sanguine specifice care să o poată diagnostica. **Prezentare de caz:** În acest raport, prezentăm cazul unui pacient în vârstă de 51 de ani, cunoscut cu tulburări mentale și comportamentale datorate abuzului de alcool, cirozei hepatice, encefalopatiei hepatice precoce, anemiei și hipoproteinemiei, care s-a prezentat la departamentul de dermatovenerologie a clinicii noastre cu drept prinicipală acuză a unui hematom la nivelul articulației cotului, survenit în urma unei agresiuni fizice. În ciuda îngrijirilor medicale, statusul pacientului s-a deteriorat semnificativ, cu extinderea semnificativă a eritemului, o hiperpigmentare profundă și extinderea sensibilității. În consecință, pacientul a fost transferat în regim de urgență la departamentul de chirurgie plastică, cu suspiciunea de fasceită necrozantă, drept diagnostic.

Concluzie: Cunoscând faptul că infecțiile necrotizante ale țesuturilor moi au un caracter acut, agresiv și rapid progresiv, în cazul pacienților cu ciroză hepatică acestea apar mai des decât în cadrul populației generale, datorită fenomenului de translocație bacteriană de la nivelul lumenului intestinal - consecință a creșterii bacteriemiei, a permeabilității crescute și a scăderii motilității intestinale. Excesul bacteriilor intestinale este, de asemenea, responsabil pentru apariția hiperamonemiei, ducând, în consecință, la apariția encefalopatiei hepatice. Ținând cont de toate acestea, complicațiile apărute fac ca acest grup de pacienți să prezinte un risc crescut de mortalitate.

Cuvinte-cheie: fasceită, ciroză, encefalopatie, necroză, translocație bacteriană

THE GENETIC AND ACQUIRED HOST SUSCEPTIBILITY TO NON-TUBERCULOUS MYCOBACTERIAL INFECTIONS

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ABSTRACT

Non-tuberculous mycobacteria (NTMs) are ubiquitous, non-pathogenic microorganisms that produce lung/ pulmonary, extrapulmonary and disseminated infections in people with immune deficiencies. If lung diseases with NTM (LD-NTM) occur mainly in immunocompetent individuals with pre-existing respiratory diseases, extrapulmonary and disseminated infections occur only in immunocompromised hosts. Both local risk factors and systemic risk factors are of particular importance in the pathogenesis of NTM infection. Genetic susceptibility plays an important role in the pathogenesis of extrapulmonary infections and disseminated with NTM, while acquired susceptibility plays an important role in lung infections, but also extrapulmonary and disseminated with NTM. Recognition of these susceptibilities has a decisive role both in establishing the diagnosis of NTM infection and in appreciation of appropriate therapeutic conduct, especially in patients with genetic susceptibility.

Key words: NTMs, genetic and acquired host susceptibility, host risk factors

INTRODUCTION

The incidence of NTM infection is increasing worldwide [1]. The increase in life expectancy and the higher incidence of this infection in the elderly segment of the population have fully contributed to this fact [2]. Also, the number of immunosuppressed patients of all ages is constantly increasing due to the increased incidence of HIV/ AIDS, but especially due to medical advances in treating various autoimmune diseases and the availability of organ transplants as a method of treating various serious diseases [3].A step forward in the management of these infections was the improvement of diagnostic techniques for NTM infection [4]. On the other hand, the diagnostic difficulties generated by the colonization with NTM of various biological samples and the total ignorance of the predisposing factors of these infections led to a more difficult management of this disease [5]. In addition, given the difficulty of eradicating NTM and its considerable recurrence, the identification of suitable candidates for treatment and the timing of initiation of therapy are also challenging [6]. Given that, in many developed countries, the incidence of NTM infection exceeded the incidence of tuberculosis, it is necessary to better understand the pathogenesis of this disease, namely to understand which are the population risk groups that develop these infections [7]. If NTM-LD occurs predominantly, in patients with pre-existing organic lung disease (COPD, emphysema, pulmonary fibrosis, bronchiectasis), extrapulmonary damage and the disseminated form of NTM infection most often occur in immunosuppressed individuals, through genetic or acquired causes [8].

This review summarizes the main risk factors for lung and disseminated NTM infection and indicates a strategy for identifying genetic and acquired immunodeficiencies.

GENETIC SUSCEPTIBILITY

Human leukocyte antigen alleles. Although the suspicion that tuberculosis is a hereditary disease existed before the discovery of the tubercle bacillus in 1882, with the discovery of the pathogen of tuberculosis, the importance of genetic susceptibility to this disease was largely ignored [9]. It was not until the last decades of the twentieth century that scientific evidence of the genetic predisposition to bacillary infection began to appear. The relationship between tuberculosis infection and HLA-DR2 histocompatibility antigens has been known for over 30 years [10]. There are authors who have established a link between innate susceptibility to mycobacterial infection and certain human leukocyte antigens [11], mainly those of class I of antigens A10 and B8 and of class II DR2 (DRB1 1501)[2]. For

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the first time, the involvement of the genetic factor in the spread of MAC infection in HIV-positive patients was demonstrated by Naik, who showed an association with the alleles DR2, DRB1 0601 and a negative association with the alleles DMA 0102 [12]. Subsequently, the association between the polymorphism of the classic HLA class II genes (DRB1 and DQB1) and the occurrence of disseminated MAC infection in HIV-1 positive patients was extended to the DMA and DMB accessory genes. In another multicenter study, it was shown that not only the polymorphism of genes encoding DRB1 alleles, but especially the presence of allelic combinations between DRB1 and DMA and DMB, plays an important role in the occurrence of MAC-disseminated disease in HIV-1 patients [13]. On the other hand, two Japanese study groups showed the presence of HLA antigens DR6, Dr4, A33 and A26, as common among patients with LD-MAC without pre-existing lung disease [14,15]. Also, the presence of HLA-A26 has been associated with disease progression.

Mendelian susceptibility to mycobacterial diseases (MSMD) is an immunodeficiency syndrome of genetic cause, with increased susceptibility to pathogenic and opportunistic mycobacteria in the environment [16].Only half of patients with MSMD have an identified genetic cause [17]. To date, nine genes responsible for MSMD are known [18]. Seven of these are inherited in an autosomal recessive or autosomal dominant model (-IFNR1, -IFNR 2, STAT1, IL12B, IL12RB1, IRF8 and I SG15 genes) and two are related to X (IKBKG and CYBB genes) [1]. In these patients, BCG vaccination causes a systemic and severe form of mycobacterial disease, often fatal, which is why BCG vaccination should be avoided. The most serious forms of the disease are autosomal recessive deficiencies of complete interferon gamma 1 (IFN-gammaR1) and receptor 2 (IFN-gammaR2)[19]. MSMD due to 1-IFN receptor 1 and 2 deficiency is a genetic condition with autosomal recessive and autosomal dominant transmission. The autosomal recessive form, characterized by complete receptor deficiency for -IFN, is manifested from childhood by the appearance of: disseminated forms of mycobacterial infection after BCG vaccine, severe and recurrent infections with mycobacteria or by the inability to form well-circumscribed mycobacterial granulomas [20].The diagnosis is suggested by the complete absence of receptor expression on the cell surface. The therapy of these syndromes is based on antimycobacterial treatment, because cytokine replacement therapy (-IFN) has no results [21]. The autosomal recessive form, characterized by partial -IFN receptor deficiency on the cell surface, is manifested later in childhood or adolescence by localized or disseminated infections after BCG vaccination, as well as by pulmonary or osteoarticular infections with NTM, but with retained capacity of mycobacterial granuloma formation. Multifocal osteomyelitis with NTM is a feature of these patients [22]. The diagnosis is suggested by a 3 - 5-fold increase in R1 receptor expression on the surface of monocytes. The diagnosis is confirmed by sequencing the gene encoding the R1 receptor for -IFN. Mycobacterial infections should be treated aggressively with antimycobacterial drugs, and some patients may also respond to -IFN[23].

MSMD due to deficiency of the p40 subunit of IL-12 occurs as a result of a mutation in the gene encoding the IL-12B subunit. This genetic condition, with autosomal recessive transmission, is manifested by systemic infections after the administration of the BCG vaccine, systemic infections with NTM and sepsis with Salmonella [24]. These infections can occur from childhood, but can also manifest later with localized mycobacterial infections. The diagnosis is suggested by the absence of secretion of the p40 subunit of IL-12. These infections respond favorably to the administration of antimycobacterial drugs, but also to the additional administration of cytokines (-IFN), when the therapeutic response to antimycobacterial therapies partial [25]. MSMD due to IL-12 β1 receptor deficiency is an autosomal recessive genetic disorder. Lack of expression of these receptors has the effect of decreasing gIFN secretion from lymphocytes and NK cells. This genetic condition is manifested by extent infections with NTM. After BCG vaccination, patients with these genetic defects may develop a systemic infection, and the developed tissue granulomas are well organized [26]. The diagnosis is suggested by the absence of IL-12 receptors on the surface of T lymphocytes and deficient STAT4 phosphorylation in response to IL-12 lymphocyte stimulation [27].Treatment of NTM infections in these patients should be performed with antimycobacterial antibiotics and cytokines (-IFN), as the gIFN receptor and downstream stimulation are intact [28].

MSMD due to STAT1 deficiency is a genetic disorder with autosomal dominant and autosomal recessive transmission, which occurs as a result of a mutation in the gene encoding STA1 synthesis, which is a key translator of the IFN type II (-IFN) signal and IFN type I (α/β-IFN)[29].The autosomal dominant form, characterized by altered STAT1 dimer formation following -IFN induction, results in a defective -IFN signaling, but with an intact α/β-IFN-mediated antiviral defense. Patients with this genetic defect who survived mycobacterial infections in childhood have reached adulthood. The autosomal recessive form (homozygous), characterized by altered signaling in both IFN type I and IFN type II, is manifested by mycobacterial infections from the first year of life, fatal, these patients being ideal candidates for stem cell transplantation [30].

Heterozygous mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene manifests phenotypically through CF manifestations such as: chronic pancreatitis, sinusitis and bronchiectasis [31] and over 25% of the patients with CF have evidence of NTM infection (LD-NTM) [32]. In case of CF patients isolated NTM lung disease (NTM-LD) occurs more often. In CF patients the species varies between geographical regions with M.avium complex (MAC), M. kansasii and M.abcessus being the most common in the United States and M.abcessus being the most frequent in Europe [33]. There is also a reported transmissibility of M.abcessus from one patient to antother. A study by Kim showed that patients with a single mutation of CFTR have greater progression of radiological lesions [33], while other authors have shown that the nodular-bronchial form appears in those patients with CF effusion[34]. Zledalski evaluated 50 patients with idiopathic bronchiectasis

or NTM-LD (20 patients) for CFTR mutations and performed iontophoresis, resulting 50% being homo or heterozygous for the CFTR gene and 20% met the HR criteria[35]. A 24% incidence of at least one CFTR gene mutation in the patients with NTM-LD was showed in another report [36]. Jang et al [37] investigated the relationship between CFTR mutations and the susceptibility to NTM lung infection in a Korean population, showing that CFTR genes variants can increase risk to NTM infections and Q1352H mutation is significantly higher in in NTM patients in the Korean population. Mutations in the CFTR gene are more common in Europeans [38], and tend to become more frequent as other rare alleles of CFTR are added to the more than 1500 mutations already known [39]. In a study by Bar-On to asses NTM prevalence in CF patients, M.abcessus was the most prevalent NTM and caused prolonged infection despite therapy [40].

There are few articles on the association between mutations in the gene encoding **α-1 antitrypsin (AAT) and NTM-LD**. Some phenotypic conditions predispose to NTM lung disease, such as emphysema and bronchiectasis. AAT prevents emphysema, bronchiectasis and protects the host against NTM infection. Also it enhances macrophage immunity against NTM [41]. Heterozygous AAT mutations are more often found in patients with NTM-LD compared to general population [42]. Kim and colleagues compared the tomographic aspects of NTM-LD in patients with normal and pathological phenotypes of AAT and found no differences in the diagnosis or follow-up [3]. Kaminska et al. studied patients with bronchiectasis and lung infections with rapidly growing NTM (RG-NTM) resulting that 27% had anomalous AAT proteins while M.abcessus was isolated in 64% of patients [43]. It remains unclear whether AAT mutations predispose to NTM- LD- independent of other respiratory diseases associated with AAT deficiency [44].

Natural resistance-associated macrophage protein 1 (NRAMP1) or SLC11A1 is an iron ion transporter that is located in the lysosomal membrane during phagocytosis [45] with evidence from the literature data for an association between some polymorphisms of SLC11A1 and NTM-LD. Koh et al [46]compared the prevalence of three polymorphisms of the SCL11A1 gene in patients with type 2 NTM-LD (MAC and M. abcessus) vs control subjects resulting that heterogeneity of some exon regions of codon 543 has been associated with an increased risk of NTM-LD. However, initial studies by Huang et al [47] found no associations between SCL11A1 gene polymorphism and NTM-LD and a meta-analysis found no association between SCL11A1 and tuberculosis (TB) [48]. Sapkota et al [49] investigated the genomic structure of SLC11A1 and the association with MAC infection in a Japanese population and observed that rs2279014 T allele is an SNP exhibiting protection against the infection.

Vitamin D has well-expressed receptors on the surface of lymphocytes and macrophages that exert immunomodulatory effects, by suppressing the production of -IFN and IL-12 that promote the proliferation and activation of Th2 lymphocytes more than that of LTh1[50]. LTh1 is currently thought to produce cytokines that promote granuloma formation and mediate cellular immunity, while LTh2 mediates humoral immunity[51]. The imbalance between Th1 and Th2 responses has been described in many immunological and infectious diseases, including diseases such as asthma [52,53], atopy [2], ulcerative colitis[54], pulmonary tuberculosis [55], leprosy and leishmaniasis [56]. Starting from the finding that vitamin D receptor (VDR) polymorphism is associated with the risk of TB [57], it has been shown that the polymorphism of this receptor can also be associated with NTM-LD susceptibility. The VDR gene is located on chromosome 12q13 and has 4 classical nucleotide polymorphisms, FokI, BsmI, ApaI and TaqI, which have been extensively studied and have been reported ***to*** affect risk in various pathologies. The FokI site is a functional polymorphism of the VDR gene that can alter the amount of VDR produced and correlates with the plasma levels of vitamin D in patients with TB [58]. Numerous epidemiological studies have suggested that low levels of vitamin D in the host are associated with increased susceptibility to TB [59,60,61]. Vitamin D deficiency is also associated with extrapulmonary dissemination in TB, which indicates that vitamin D status is associated with the disease phenotype [62]. In this regard, Geder et al. [63] tested 56 patients with M.malmoese lung disease for three types of receptor polymorphisms and identified a lower prevalence of Flavobacteriumokeanokoites I (FokI) polymorphism while the prevalence of Acinetobacterpasteuranis, subspecies IA and Thermusaquaticus YTI (TaqI) subspecies was increased. However, Tanaka et al [64] did not find any difference for FokI and TaqI polymorphisms in sick people compared to healthy controls.

The 2 toll-like receptor (TLR2) has the role of mediating the recognition of bacterial antigens by the innate immune system [65]. This receptor is recognized as the receptor by which macrophages recognize mycobacteria [66]. A study by Ryu et al. [67] which included 17 patients with NTM-LD (MAC, M. abscenssus), measured mRNA production for TLR2, TNF, Il-12, α-TNF and the p40 subunit of Il-12 in blood monocytes. The level of these substances after exposure to MAC was lower compared to the level produced by monocytes in healthy subjects. Thus, exposure to MAC in the presence of anti-TLR2 antibodies reduces the production of mRNA and cytokines in controls compared to patients with NTM, suggesting a TLR2 defect that confers NTM susceptibility to patients. TLR2 is present in macrophages, dendritic cells and lung epithelial cells. Expression of phosphatidyl-myo-inositol surface molecules (PIMS) on M.abscessus stimulates the immune response of macrophages via TLR2 [68]. Stimulation of TLR2 also results in the release of IL-8 [69]. Certain variants of M. abscessus also stimulate the release of TNF-α following interaction with TLR2 [70].

ACQUIRED SUSCEPTIBILITY. OBSTRUCTIVE PULMONARY DISEASES (ASTHMA, COPD)

Local conditions in obstructive pulmonary diseases like COPD and asthma, such as impaired clearance of secretions, abnormal composition of airways surface liquid, abnormal

composition of sputum and airway damage caused by persistent inflammation could increase the risk for association with NTM [71]. In COPD, parenchymal destruction may be the factor that impairs clearance of inhaled pathogen. In asthmatic patients, bronchial hyperresponsiveness may impair mucociliary clearance [72]. In 2016, Marras and al [72] described a high association between obstructive diseases and NTM pulmonary disease in an over 6 million people study (COPD and asthma were associated with approximately nine-fold and five-fold higher adjusted incidences of NTM-LD). The same high association rates were found in 2013 by Andrejak and al.[73] in a study on 332 Danish patients with NTM infection. The risk infection rate in these subjects had an odd ratio (OR) of 15.7 (95% CI 5.2–11.6) for NTM infection among patients with COPD, a higher OR of 29.1 for COPD patients receiving inhaled corticosteroid therapy and an OR of 7.8 in asthma patients, compared to the general population. A recent study [73] revealed that NTM pulmonary disease is associated with nodular/bronchiectatic changes on chest CT in 47% of cases and with the form of infiltrative/cavitary in 35% of cases. According to microbiological data in the same study, M. avium was present in 39% of the examined population, the most frequently strain associated with obstructive pulmonary diseases. M. gordonae is the only strain without association with NTM lung diseases. Marras and al [72] described the presence of M. xenopi and M. avium complex (MAC) and founded higher rates of cavitation in patients with M.xenopi than at patients with MAC (46% vs. 16%; p = 0.01). M. xenopi was associated with COPD and death risk. The high mortality risk for patients with COPD and NTM infection was found by Pyarali and al [74] to be 1.43 times higher compared to uninfected COPD patients. There are studies [75] that described the association between inhaled corticosteroid use and risk of pulmonary NTM, taking in account that the local immunity may be compromised by ICS therapy. There is risk associated with dose and type of inhaled corticosteroid and also with the systemic corticosteroid use [76]. Balavoine and al [77] underlies the risk of association between chronic macrolide treatment in COPD and the selection of the resistant NTM.

BRONCHIECTASIS

The pulmonary infections of **non-CF bronchiectasis** due to NTM are increasing worldwide. In a study performed by Mirsaeidi et al on 182 patients with non-CF bronchiectasis and NTM lung infection, NTM was isolated in 68 (37%) of patients and the most common was M. avium complex (MAC) [78]. In a study performed by Koh WJ et al to determine the frequency of NTM lung infection in patients with bilateral bronchiectasis and bronchiolitis in CT findings, NTM lung infection was isolated in 36 (34%) of 105 patients, with the most common isolated organism being MAC in 50% of the patients [79]. One pathophysiological mechanism involved in NTM lung infection in the patients with bronchiectasis is the adhesion of the NTM to the damaged areas of the respiratory mucosa. Fibronectin attachment protein from NTM binds to the one from the extracellular matrix from the damaged mucosal surface [80]. The main clinical symptoms for the non-FC patients with bronchiectasis and NTM infection are cough, sputum production, wheezing and dyspnea. There are also some phenotypic features that are associated with NTM infection: the female gender, hence low body mass index (BMI), pectus excavatum, scoliosis and mitral valve prolapse [81]. The treatment of patients with non-CF bronchiectasis and NTM lung infection includes management of bronchiectasis with hypertonic saline or positive pressure devices in stable patients and management of the NTM lung infection [81].

Gastroesophageal reflux disease (GERD)

GERD is characterized by reflux of gastric contents, which causes various symptoms and respiratory complications. It is associated with many lung diseases, such as asthma, bronchiectasis, chronic bronchitis but also with NTM infection with the appearance of a lesion in the bronchial epithelium secondary to the reflux of gastric contents [82]. The association between NTM-LD and GERD has been reported by many authors, so Hadjiliadis [83] and Griffith [84] reported an increased prevalence of M. fortuitum and M. abscessus infection in these patients. Numerous reports have stated that GERD is a predisposing factor for NTM lung disease. However, insufficient data ***is*** available on treatment with proton pump inhibitors that suppress acid secretion and thus predispose to NTM or represent a marker of severe GERD [85].

DIABETES MELLITUS

Diabetus mellitus is a metabolic disease with countless manifestations and complications, including infections caused by NTM, which appear as complications in these patients due to the lack of clear diagnostic methods[86]. The most associated localized infections with NTM are: cutaneous produced by M.chelonae [87,88] at the site of insulin injection, pleural with MAC [89], pulmonary and musculoskeletal with M. Kansassii [90], thyroid with MAC [91], but without a clear incidence of infections in diabetic patients to date. Patients with diabetes are vulnerable to NTM infections, due to the resistance of infections to antituberculosis treatments and routine diagnostic methods are required. Early treatment will lead to a rapid improvement but also an assessment of the susceptibility of antibiotic treatment to these microorganisms [88].

CHRONIC KIDNEY DISEASE

Chronic kidney disease is a risk factor for NTM infections [92], associated with infections caused by cutaneous, peritoneal [93], bone, joint nontuberculous mycobacteria, especially slow-growing mycobacteria [94,95]. Peritonitis secondary to peritoneal dialysis is a major cause of morbidity and mortality in these patients [96]. Patients with kidney disease also have a risk of infection with NTM related to the source of contaminated water, with a high risk of peritonitis. However, M. tuberculosis is the most common pathogen for peritonitis with negative cultures, while the most common species of NTM that cause this type of infection are M. Fortuitum (39%) and M. Chelonae (14%). Both species are considered fast-growing mycobacteria, being

isolated seven days after growth [97],[98].

CANCER PATIENTS

Cancer patients are also at an increased risk of NTM diseases on one hand because of the decreased cell-mediated immunity and on the other hand because of chemotherapy. Patients with lung cancer have a particularly high risk of developing NTM infections, probably because of the damaged airways, but hematological malignancies also represent an increased risk of localized catheter-associated and disseminated infections. Hairy cell leukemia has been associated with NTM infections since 1980 [100]. Other diseases that pose a potential risk for NTM infections include granulomatous diseases, variable immunodeficiency syndrome and hypogammaglobulinemia (Table 1).The relative risk of NTM infection varies greatly depending on the pathology of the host. Thus, Henkle collected the relative risk values of NTM infection obtained by several authors, on groups of different patients depending on the pathology of the host, pre-existing NTM infection [3]. These values of the relative risk of NTM infection and the bibliographic references used by Henkle can be found in the last two columns of Table 1.

Table 1. The immunosuppressive conditions and risks for NTM infections[3]

Underlying disease or Treatment	NTM cases in included references	Lung disease-NTM	Disseminated disease-NTM	Skin/soft tissue/catheter disease_NTM	Overall Risk/Relative-Risk (RR)	References
AIDS	972	-	100%	-	24%	99
Hairy cell leukemia	9	-	100%	-	5%	100
Hematologic malignancies	34	76%	24%	-	1.2%	101
Hematopoietic stem cell transplant	97	18%	9%	70%	0.4-4.9	102,103,104
Solid organ transplant	40	50%	15%	35%	0.02-1.1/100.000	97,105
Corticosteroid therapy for immune-mediated inflammatory diseases	182	100%			RR oral: 8/100.000 RR inhaled: 24.3/100.000	71,106
Biologic therapy for immune-mediated inflammatory diseases	123	56-67%	8%	35%	74/100.000	107,108

HIV/AIDS.

Immunosuppression is a major risk factor for infections with NTM. During the earlier times of the infection with HIV, disseminated MAC infections appeared in up to 24% of AIDS patients. Nevertheless with the introduction of highly active antiretroviral therapy (HAART) in HIV patients, MAC infections have become less important, while both the increased need for organ transplants, especially stem cell transplants, as well as the more frequent use of biologicals and immunosuppressive therapies in patients with autoimmune diseases have increased the incidence of NTM infections in these patients [100]. According to new reports, inhaled steroid therapy increases the risk of NTM infections in COPD patients and probably less in those with bronchial asthma [110]. In contrast to other opportunistic infections in the context of HIV infection, disseminated MAC infection only occurs from a CD4+ cell count of less than 50 cells/mm3, which speaks for the importance of the cell-mediated immune system in the fight against mycobacteria [109],[110]. M. kansasii can also lead to disseminated NTM infections, but it is primarily responsible for lung disease in over a half of AIDS patients [111]. With the introduction of HAART therapy in 1997, an important reduction in the number of disseminated MAC infections was achieved. In addition, through the introduction of antiretroviral therapy, the "immune reconstitution inflammatory syndrome" has developed, as a result of rebuilding the pathogen-specific immune response during this therapy. In most cases, this syndrome, which is mainly associated with MAC, presents with fever and painful lymphadenitis [112]. A prophylactic treatment with 1200mg azithromycin once a week should be currently given in HIV/patients with CD4+ counts below 50 cells/mm^3. In addition to clarithromycin, ethambutol and/or rifabutin the treatment for disseminated MAC should also include antivirals to maintain the underlying immunosuppression under control [113]. A shortened time to death was seen in untreated disseminated MAC in AIDS patients, but similar survival results were also found in treated patients had a similar compared to non-MAC matched controls [3].

Iatrogenic Immunosuppression. It is already known that **glucocorticoids** increase the risk of pneumonia in COPD patients and of tuberculosis by 5-fold [114], while the relative risks for NTM infections are even higher. In a case-control study from Oregan, Washington the intake of prednisolone was 8 times higher among NTM cases compared to controls [109]. Moreover, inhaled glucocorticoids have been linked to a significantly increased risk of NTM in COPD patients in a study from Denmark [71], as well as in asthmatic patients in a study from Japan [115]. In both studies the doses of prednisolone were above 15 mg/day and fluticasone above 800 μg/day.

Biologic therapy, that target the TNF signaling pathway, like the tumor necrosis factor alpha (TNF-alpha)-inhibitors, are essential in the therapy for autoimmune diseases such as rheumatoid arthritis, psoriasis and Crohn's disease. In a study from the USA, patients receiving a new therapy with infliximab, etanercept or adalimumab had a 5 to 10-fold increased risk of NTM disease compared to patients with rheumatoid arthritis without this therapy or to the normal population. The NTM illnesses were even more common than the tuberculosis in these patients [114]. Other biological therapies that theoretically increase the NTM risk are rituximab (anti CD20 antibodies), abatacept (T-cell co-stimulation modulator), tocilizumab (anti-IL-6 antibodies) and ustekinumab (anti-IL-12), but very little safety data exists on this subject and further studies are required [114]. Unlike tuberculosis, where a benefit of screening for latent infection before starting an **immunosuppresive therapy** have been demonstrated, prospective screening for latent NTM infections has not been recommended for any of

immunosuppressed groups [111]. However, CT imaging and respiratory sampling in searching for pulmonary NTM should be considered at least in patients with chronic cough that cannot be easily explained by usual causes.If possible, discontinuing immunosuppressive drugs or their substitution with non-biological treatment such as methotrexate or second-line therapies such as abatacept or tocilizumab, which currently present as lower risk medication, increases the likelihood that antimicrobial therapy for NTM infections will be successful [112],[109]. A small study from Japan showed that NTM infections in patients with rheumatoid arthritis had a positive outcome in those patients who stopped taking the biological therapy. Furthermore fatal cases were associated in this study with underlying lung diseases such as aspergillosis and interstitial lung disease [3],[113].

Organ transplant. After an organ transplant patients must as well start an immunosuppressive therapy. The most commonly administered drugs are mammalian target of rapamycin (mTOR) inhibitor (sirolimus), calcineurin inhibitors (tacrolimus and cyclosporine), prednisolone and other drugs depending on the transplanted organ [3]. In a 2014 report that included 293 organ transplants, it was observed that the most NTM infections occurred after lung transplantation (61%). Heart and liver transplants with similar risks for NTM infections followed with 26% and 31% respectively and kidney transplants came on the last place with 17% chances for NTM disease [102]. In other three studies, MAC and M. abscessus were described as the most frequently mycobacteria in patients with solid organ transplants with 45% each [105],[115],[116].

NTM infections have also been found in patients with **hematopoietic stem cell transplantation** (HSCT) in the treatment of hematological cancers such as leukemia or multiple myeloma. HSCTs are most frequently associated with catheter and blood infections, but 18% (17/97) were associated with pulmonary NTM in a 2014 review in NTM-infected HSCT-patients. Graft versus host disease was an important risk factor, present in 46% of NTM infections and corresponding with an increase in immunosuppressive medications [103]. However, death related to pulmonary and disseminated NTM disease were in the literature in 7/94 (7.5%) of HSCT patients reported. Overall, the most frequently NTM in HSCT patients were MAC, *M. abscessus/M. chelonae* and *M. haemophilum* [117],[118],[119]. Therapy in transplanted patients is difficult because of the interactions between rifampicin, macrolides and aminoglycosides with calcineurin inhibitors and tacrolimus, and antibiotics should therefore be chosen very carefully. There have been some reports of graft vs host reactions in patients who have reduced immunosuppressive therapy in order to achieve a better immune system function. This risk should be included in the risk-benefit assessment in the fight against the mycobacteria [3].

In **conclusion**, knowing the host risk factors to NTM infection helps to establish the correct diagnosis and allows the appropriate choice of therapy for lung and disseminated NTM infection.

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SUSCEPTIBILITATEA GENETICĂ ȘI DOBÂNDITĂ A GAZDEI LA INFECȚIILE MICOBACTERIENE NON-TUBERCULOASE

REZUMAT

Micobacteriile non-tuberculoase (MNT) sunt microorganisme omniprezente, nepatogene, care produc infecții pulmonare, extrapulmonare și diseminate doar la persoanele cu deficiențe imune. Dacă infecțiile pulmonare cu NTM apar, în principal, la persoanele imunocompetente cu boli respiratorii preexistente, infecțiile extrapulmonare și diseminate apar doar la gazdele imunocompromise. Atât factorii de risc locali, cât și factorii de risc sistemici sunt de o importanță deosebită în patogeneza infecției cu MNT. Sensibilitatea genetică a gazdei joacă un rol important în patogeneza infecțiilor extrapulmonare și diseminate cu MNT, în timp ce susceptibilitatea dobândită a gazdei joacă un rol important în infecțiile pulmonare, dar și extrapulmonare, precum și diseminate cu MNT. Recunoașterea acestor susceptibilități are un rol decisiv atât în stabilirea diagnosticului de infecție cu MNT, cât și în aprecierea conduitei terapeutice adecvate.

Cuvinte cheie: MNT, susceptibilitatea genetică și dobândită a gazdei, factorii de risc ai gazdei.