Founded 1897 • New Series

Vol. CXXII • No. 1/2019 • April

Romanian Journal of Military Medicine

REVISTA DE MEDICINĂ MILITARĂ

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- *Primary retroperitoneal tumours – a brief classification and differential diagnosis*
- *SGLT2 inhibition in patients with type 2 diabetes and cardiovascular diseases: which are the benefits*
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Romanian Journal of Military Medicine (RJMM) is included in Romanian College of Physicians Medical Publications Index.

www.revistamedicinamilitara.ro

Romanian Journal of Military Medicine, New Series, vol. CXXII, No 1/2019, April
ISSN-L 1222-5126; eISSN 2501-2312; pISSN 1222-5126

RJMM
Romanian Journal of Military Medicine

CLARIVATE ANALYTICS EMERGING SOURCES CITATION INDEX INDEXED

Founded 1897 • New Series
Vol. CXXII • No. 1/2019 • April

Edited by the Romanian Association of Military Physicians and Pharmacists.

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ROMÂNIA
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Atestat

Ministrul apărării naţionale

dorind a răsplăti meritele

redacţiei pubilcaţiei „Revista de Medicină Militară",

în semn de apreciere a contribuţiei avute la creşterea prestigiului structurilor medicale din Armata României, cu ocazia aniversării a 120 de ani de la înfiinţare,

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-cu însemn de pace-

Acordat în Bucureşti, 15 Noiembrie 2017

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Atestat E.O.A.R./nr.1

Nr.crt. 21

EDITORIAL

Academician Eugeniu Gh. Proca, founder of modern urology school in Romania. A memory

Gral (R) Prof DAN MISCHIANU

Chief of Urology Clinic, Carol Davila University Central Emergency Military Hospital Faculty of General Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Dan Mischianu

Motto: "*You only live once – but if you work it right, once is enough*." Joe E. Lewis

The history of Romanian Surgery will record, I am absolutely convinced, the name of Academician Eugeniu Gh. Proca (January 12, 1927 Godeni Muscel - 07.03.2004 Bucharest). As he testified in a televised interview, he wanted to become a general surgeon, he did not like urology at first, but in the end, as it happens in life, "you love who you live with." [1]

Image 1: Academician Eugen Proca at the age of great responsabilities and professional fulfilments

He didn't regret at all the fact that he became a Romanian urologist, transforming the Romanian urology to which he added new development opportunities; therefore the title of this article is perfectly true. Moreover, after many years, I can see that his first love – general surgery –seems to disappear as a stand-alone specialty, slowly but surely…

Urological surgery is a noble, beautiful, difficult and extremely consuming profession. As one of my colleagues, a surgeon from Constanta – Professor Vasile Sârbu – wrote: "The Romanian surgery amazed through two miracles. One of them happened at the end of the nineteenth century when, in two to three decades, starting from almost nothing, it came to the level of the brightest surgical schools in the world. The second miracle took place in the twentieth century and it refers to the ability to improvise, to do great surgery in all areas, under dramatic conditions, with instrumental, material and technology shortcomings. "[2] Perhaps the chance of Romanian surgeons was the fact that in the twentieth century the three Ministers of Health, who were surgeons, proved an exceptional organizational spirit in their mandates, (Acad. N. Hortolomei 1939-1940, Prof. Dr. Voinea Marinescu 1954 -1966 and Acad. Eugen Proca 1978-1985). I do not mention the name of Prof. Constantin I. Angelescu, a surgeon and a professor at Brâncovenesc Hospital and then at Filantropia Hospital because his ministerial mandate – excelent in substance! – took place at the Ministry of Public Works 1914-1916 and at the Public

Instruction.

Image 2. At the Hospital Prof. Dr. Th. Burghele (previously called Panduri) near his colleagues: primary urologists and secondary urologists

Having all the available means of information from the present, in which we all become when we want and especially when it is not the case, curious "researchers" in the biographies and in the personal identification numbers of everyone from everywhere, I do not want to present the commonly known things regarding the life and work of an overwhelming man, such as Acad. Eugen Proca, I only want to reveal some rather special information.

The professor was born on January 12, 1927, in Godeni, from the former county Muscel. He was a "trueborn inhabitant of Muscel", the only son of Gheorghe C. Proca and Maria Proca. The people from Muscel, who lived close to the mountains, are known to be fiery and combative spirits (see "partisans from Muscel"). Brilliant graduate of "Dinicu Golescu" High School in Câmpulung Muscel, he becomes a student of the Faculty of Medicine in Bucharest in 1945 and at the same time, a student of the Medical-Military Institute. This educational ambivalence has almost always been a solution for many skilled children coming from families with modest material conditions.

Image 3. Standing beside Acad. L. Gherasim and Gl. Prof. Dr. Doc. Iuliu Şuteu

I will tell you something that I saw with my eyes and I heard with my ears a long time ago. A very long time ago...

I was a student of the Medical-Pharmaceutical Institute (currently University of Medicine and Pharmacy Carol Davila, Bucharest) and a Corporal student at the Medical-Military Institute. By the kindness of the Party Secretary of the Medical-Military Institute, as I was a student with honorable academic achievements, in the summer session 1974-1975, I was allowed to prepare the exams to come, late at

night, in a room which was more protected against the ubiquitous noise in the area of the North Railway Station. It was then that I discovered the catalogues of former graduates, in the room called "IMM Museum". There were large, thick-tatted books in a dusty cabinet. This is how I browsed, with curiosity, the catalogues of promotions of future military doctors, in which the high school photo was attached to each student. The promotions of 1940 and 1942 showed me the young and confident faces of the future generals and surgeon professors – Iuliu Suteu and Traian Oancea, once emblematic people for the Central Military Hospital. Looking at the pages which turned yellow over time, I was contradicted to find out at some point (it was midnight), the name Eugene Gh. Proca, Godeni, Muscel 1927 January, who entered the Medical-Military Institute in 1945 and below, across the whole sheet of paper, it was written with a red, thick pencil, I have such a vivid recollection of the word: "EXPELLED!".

Image 4. Minister Eugen Proca beside some of his collaborators

Years passed incredibly fast for all of us and while I was a secondary physician, studying my second specialty – urology, during a short break between two surgeries, I told the Professor what I had discovered 20 years before. We were living in difficult times, quite similar to the present ones, but I saw his unblinking blue-grey eyes shining and he even told me the story which was also mentioned by Dr. M. Mihailide in the footnotes of one of his books [3]. He said he had been expelled from the institute because he had lit the fire in a stove using the newspaper published by the party – “Scanteia” newspaper. He also mentioned the way in which, on a Sunday from April 1961, being on duty at the hospital, he took care with full empathy and unaltered human kindness of his former faculty colleague, who needed dialysis. He was uremic and only dialysis prolonged his life.

His faculty colleague was the one who had informed the State authorities about the fire lit in the stove!

Academician Eugen Proca was a Christian orthodox who believed in life after death and who, I am sure, is looking at us with empathy from up there. Academician Eugen Proca is not only the creator of the modern urology school from our country. He is and will always be an emblematic figure for Romanian surgery.

For as long as I was around him, in my perception he was a true "Imperator", intelligent to extreme, self-exigent, caustic with cheaters, tenacious, with a huge culture, always eager to self-improve and aware, at all times, that a surgeon's job does not have a program.

Anyone using a computer can easily find out about his scientific and clinical achievements: performing the first hemodialysis – April 7, 1959, World Health Day – at the hospital raised by the Professor or Academician Th. Burghele, performing the first kidney transplant in Romania in February 1980 (the operation for which he had been mentally and spiritually prepared for a very long time) and about his paideic model. What almost nobody knows is that when he was the second Minister of Health as term of office (7 years), he used to work 24/7. At six o'clock in the morning he was examining patients. Then he went in the surgery room. At 11.00 a.m. – 12.00 p.m., he went to his office from the Ministry where he tried to solve many problems of

that time: the birth issue (which is now of almost no interest to anyone), the introduction of medical promotion contests and many more, and in the evening, at 8.00 p.m. – 9.00 p.m., he returned at the hospital to see his patients...

Apparently, Ceausescu's wife was very proud of Minister Prof. Dr.Proca saying: "He is our only Minister who actually works."

The fact that the name of Academician Professor Dr. Eugeniu Proca, 15 years after passing into a better and fairer world, is not found in the memory of some of our institutions, is an additional proof that the Latin parable "Nemo propheta in patria sua " (Luke's Book, Chapter IV, verse 24) or in English translation: "No one is a prophet in his country" remains forever and, unfortunately, perfectly true!...

It seems that Oscar Wilde also enlightened our understanding with the following aphorism: "the truth is rarely pure and never simple…"

I dared to reveal something from my own truth about the work and life of this Man.

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REVIEW ARTICLE

Article received on September 30, 2018 and accepted for publishing on February 1, 2019.

Primary retroperitoneal tumors – A brief classification and differential diagnosis

Alexandru Cherciu[1], Lucian Iorga[1], Radu Anghel[1], Dragos Marcu[1,2], Raluca S. Costache[1,2], Ioana Bratu[1], Maria Manea[1]

Abstract: *According to literature the primary retroperitoneal malignant pathology is rare, representing less than 0.5% of all malignancies. In spite of its low incidence, this pathology often proves challenging in terms of diagnosis and treatment. The most com¬mon retroperitoneal malignancy is represented by lymphoma that accounts for approximately 33 % of the retroperitoneal malignancies. Other frequent retroperitoneal tumors are sarcomas: liposarcomas, leiomyosarcomas and malignant fibrous histiocytoma (in order of incidence). We have analyzed the existing data regarding the most common primary retroperitoneal tumors in order to realize a brief classification and their differential diagnosis considering their clinical, imagistic, histopathological and molecular characteristics.*
The technological developments that have been made over the years in terms of imaging investigations, as well as in biomelecular and cytogenetic studies have offered new possibilities for of assessing a retroperitoneal mass in order achieve more precise informations that can guide physicians to better distinguish between different types of retroperitoneal tumors and therefore their therapeutical protocol.

Keywords: *retroperitoneal tumors, diagnosis, imaging investigations*

INTRODUCTION

According to literature, approximately 70% – 80% of the primary retroperitoneal neoplasms it is estimated that are malignant, these tumors accounting for 0.1%–0.2% of all malignancies [1, 2]. These retroperitoneal masses can be divided into solid and cystic tumoral masses [3-5]. Because the treatment options vary, it is useful to be able to distinguish these masses and to diagnose them clearly in order to choose the correct therapeutical protocol. Imaging investigations and biopsy play a crucial role in establishing the tumors characteristics in terms of tumour content, size, shape, wall thickness, the presence of intratumoral septa, calcification, fat, necrosis, detecting the extension of the disease in nearby structures and also distant metastases, as well as in predicting the patients prognosis [1, 6, 7].

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SOLID RETROPERITONEAL TUMORS

Solid retroperitoneal neoplasms can be split into four groups: 1. mesodermal neo-

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plasms; 2. neurogenic tumors; 3. germ cell, sex cord, and stromal tumors; 4. lymphoid and hematologic neoplasms [8].

Mesodermal Neoplasms

Retroperitoneal neoplasms are predominantly of mesodermal origin, the most frequent tumors located in the retroperitoneum being liposarcomas, leiomyo-sarcomas and malignant fibrous histiocytoma.

Retroperitoneal sarcomas represent approximately 0.2% of all malignancies, being more frequently discovered during the 6th decade of life. Quite often these retroperitoneal masses are found incidentally during imaging investigations, especially in early disease stages when the patient has no alarming sings, nor symptoms that could alert the patient and raise a question mark. Otherwise, a great percentage of retroperitoneal tumors are detected in advanced stages, when the tumor has important dimensions, often being associated with symptoms related to the nearby organ compression or invasion and therefore being less possible to achieve a complete and oncological surgical resection. The rate of relapse in such cases is high, often these patients will develop distant metastases, more frequently in the lungs, liver, bones and brain [1, 2].

Liposarcoma

Liposarcoma is the most common soft tissue sarcoma encountered in adult patients.This fatty cell tumor usually develops in middle-aged individuals as a mass of submucosal tissue that grows easily without pain and ulceration, yet some lesions can grow rapidly and become ulcerated. The most recent classification of sarcomas identifies five categories of liposarcomas: well differentiated, with adipocytic, sclerosing, and inflammatory subtype, dedifferentiated, myxoid, round cell, pleomorphic.

The histological type appears to be closely related to the anatomical distribution, so the well-differentiated liposarcoma appears more frequently in the soft tissues of both limbs and retroperitoneum, whereas the mixoid/round-cell liposarcomas and the pleomorphic liposarcomas have a striking predilection for limbs.

The tumor usually grows slowly over time, leading to progressive and diffuse abdominal distention and to other symptoms that appear to be secondary to the adjacent organ compression. Physical examination can prove itself to be inconclusive (especially in early stages when the tumor is small and without secondary signs nor symptoms), but in advanced stages (when the tumor has important dimensions) it may prove to be a useful tool in terms of raising the suspicion of a retroperitoneal mass.

Laboratory studies have demonstrated that cytogenetics have an important role in diagnosing lipomatous tumors based on the differences of chromosomal abnormalities between tumor types. The lipoblastoma usually exhibits modifications of bands 8q11-13 and the gene PLAG1 has been involved as the target of chromosomal changes [9].

Another important method used for evaluating these types of tumors is represented by imaging investigations. For example, computer tomography (CT) scanning has proven to be superior to magnetic resonance imaging (MRI) when we are investigating a bone erosion and demineralization, whereas MRI provides information's about the limbs characteristics, describing the fatty nature of the tumor. Liposarcomas usually have well-defined and mostly lobulated margins. The well-differentiated liposarcomas are formed of mainly fat tissue with septa or nodules. Myxoid liposarcomas are described as having a pseudocapsule with a homogeneous or heterogenous aspect. Pleomorphic tumors have a markedly heterogeneous structure. Another imagistic technique that can be used for the diagnosis process is angiography. This invesigation can demonstrate tumor malignancy on the basis of a prominent vascular component, therefore having an important value in planning the therapeutical protocol. The diagnostic procedure for liposarcoma is biopsy. For large (>3 cm) and deep tumors, the diagnosis implies open incisional biopsy accompanied by definitive resection. Fine-needle aspiration (FNA) should be followed by immunohistochemical and histological examination [10, 11].

Immunohistochemical examination is used for excluding other types of sarcoma. Lipid staining may

be helpful, although oil red O stains or Sudan black are insufficient for diagnosis. Helpful stains include the following [12]:

• S-100 – Positive results in fat cells and lipoblasts;

• Alpha-1-antitrypsin – Positive results in malignant fibrous histiocytomas;

• Desmin – Positive results in leiomyosarcomas;

• Myoglobin – Positive results in rhabdomyosarcomas;

• Adipophilin stain to document lipid content in the cytoplasm of the neoplastic cells using formalin-fixed paraffin-embedded issue.

Histologically the identification of lipoblasts is decisive for the diagnosis of liposarcoma. A lipoblast is capable to produce and accumulate non–membrane-bound lipid within its cytoplasm. The most important morphologic features in diagnosing the liposarcoma are the demarcated cytoplasmic lipid that shifts the indentations in an irregular hyperchromatic nucleus and the characteristic scalloping of the nuclear membrane.

Well-differentiated liposarcomas usually contain a predominance of mature fat cells with relatively few, widely scattered lipoblasts. In the sclerosing subtype of a well-differentiated liposarcoma, collagen fibrils that encircle fat cells and lipoblasts make up a prominent part of the matrix.

Myxoid liposarcoma, the most common type, is diagnosed by the observation of a delicate plexiform capillary network that is associated with both primitive mesenchyme-like cells and a variable number of lipoblasts. In the round-cell type, lipoblasts are interspersed among sheets of poorly differentiated round cells.

Poorly differentiated pleomorphic liposarcoma is recognized by a mixture of bizarre, often multivacuolated lipoblasts and atypical stromal cells, many of which contain highly abnormal mitotic figures. Hemorrhagic and necrotic areas are common. Lipoblasts are present [12].

Leyomiosarcoma

Leiomyosarcoma derives from smooth muscle tissue, Wolffian duct remnants or blood vessels, being the second most frequently primary retroperitoneal tumor. This type of sarcoma can grow and reachenormous sizes (>10 cm), being more frequently encountered in female patients, usually in the 5th or 6th decade of life. Histopathologically, it is characterized by large areas of cystic degeneration and necrosis, but calcification is uncommon. Immunohistochemistry (IHC) experimental tests have showed that the tumor cells were significantly stained by desmin and actin antibodies, with some variation in staining intensity in different tumor cells. Relatively frail vimentin staining has been observed, especially if it is compared with the intense vimentin - specific decoration of endothelial cells and fibroblasts. Leiomyosarcoma can manifest itself predominantly as extravascular (62%), intravascular (5%) in the retroperitoneum or it can have a combination of extra- and intravascular components (33%). At CT examination, small tumors are usually homogeneously solid, although large tumors have areas of necrosis and occasional hemorrhage.

Extremely rare, leiomyosarcoma can appear as a cystic tumor. The MRI highlights these tumors to have intermediate to low signal intensity on T1-weighted images and intermediate to high signal intensity on T2-weighted images, depending on the amount of necrosis. So in conclusion, the existence of necrosis in a retroperitoneal tumor, with contiguous involvement of a vessel, is suggestive for leiomyosarcoma. Distant metastases usually occur late in the course of the disease, the most common locations being the liver, the lungs and/or lymph nodes [1, 13].

Approximately 6% of these retroperitoneal tumors arise from the inferior vena cava (IVC) [1]. These tumors have a large extravascular component that makes it difficult to distinguish them from a secondary involvement of the IVC. The most commonly affected location is the segment between the diaphragm and the renal veins. Tumors involving the superior segment of the IVC (above the hepatic veins) may manifest with Budd-Chiari syndrome, those of the middle segment (between the hepatic and renal veins) manifest with right upper quadrant pain, discomfort, nephrotic syndrome, or arterial hypertension [14-17]

and the tumors of the inferior segment of the IVC (below the renal veins) can manifest with pain and lower extremity edema [13]. The differential diagnosis includes benign thrombus, angiosarcoma and a tumor extending to the IVC from adjacent organs [13, 18]. Tumors that develop from the middle segment of the IVC seem to have a better prognosis than those that arise from other IVC segments [18].

Malignant Fibrous Histiocytoma

This type of tumor is the third most common retroperitoneal sarcoma, but overall is the most frequently sarcoma in the body [19]. Malignant histiocytoma derives from primitive mesenchymal elements and it is estimated that approximately 15% of these tumors appear in the retroperitoneum [1]. In terms of gender distribution is it reported that male patients have higher risks for developing this type of tumor (75% male patients versus 25% female patients), being discovered especially in the fifth decade of life. Imaging techniques are nonspecific and usually describe a large, intrusive and heterogeneously enhancing mass with large areas of necrosis and hemorrhage, with invasion of the nearby organs, like other pathologies look alike [20]. Different patterns of calcification can be seen in the peripheral areas of these tumors. A cystic variant with peripheral calcification has been reported. The presence of calcification may help to distinguish leiomyosarcoma from malignant fibrous histiocytoma [1].

Germ cell, sex cord, and stromal cell tumors

Primary extragonadal Germ Cell Tumors

Even though germ cell tumors are present commonly in the testes or ovaries, a slight percent of germ cell tumors arise from an extragonadal location. These tumors are supposed to appear from abnormal primordial germ cell remnants that are cause of: germ cells distributed physiologically to liver, bone marrow, and brain or faulty migration of germ cells from the yolk sac or endoderm to the urogenital ridge [21]. Before an analysis of primary extragonadal germ cell tumor is made, a metastasis should be excluded. Sometimes the primary gonadal tumor could not be detectable because tumor relapse has left only a scar, with asynchronous or synchronous metastasis. This type of tumors is more frequently found in men. From the histopathological point of view, these tumors are classified in nonseminomatous germ cell tumors or seminomas, which involve teratoma, yolk sac tumor, choriocarcinoma, mixed germ cell tumors, and embryonal carcinoma. Increased levels of the beta subunit of human chorionic gonadotropin (choriocarcinoma) and of alpha-fetoprotein (yolk sac tumors, embryonal carcinoma) may be found with laboratory tests [21].

Extragonadal germ cell tumors are frequently seen near the midline, especially between the T6 and S2 vertebrae, the retroperitoneum being the second most common site after the mediastinum, a midline mass being more evocative for a primary extragonadal germ cell tumor than of metastasis. At imaging, the data collected are nonspecific for primary extragonadal germ cell tumors. The imaging characteristics are similar to those of gonadal germ cell tumors. Seminoma is occasionally located in the retroperitoneum and it can be recognized as a lobulated, large, well-defined homogeneous solid mass with fibrous septa and ring-like or speckled calcification. Non-seminomatous germ cell tumors are illustrated as heterogeneous tumors with areas of necrosis, hemorrhage and heterogeneous enhancement. The tumors in the extragonadal sites have a worse prognosis than that of their gonadal counterparts [22].

Lymphoid and Hematologic Neoplasms

The most common retroperitoneal malignancy is represented by lymphoma that accounts for approximately 33% of the retroperitoneal malignancies [1].

Lymphoma can be generally categorized into non-Hodgkin lymphoma and Hodgkin lymphoma. Hodgkin lymphoma usually occurs in patients during their second and sixth decade of life, having a bimodal age of distribution, often involving the spleen and mediastinum, manifesting itself with limited disease. Non-Hodgkin lymphoma is manifested in the forth-seventh decade of life, often associating extranodal disease in the spleen, bowel or liver. It is estimated that paraaortic lymph nodes are affected in 55% of the

patients with non-Hodgkin lymphoma, as well as in 25% of the patients with Hodgkin lymphoma. A retroperitoneal mass can be discovered in approximately 15% of the subjects with non-Hodgkin lymphoma. Mesenteric lymph nodes are also frequently involved in non-Hodgkin lymphoma [1, 2].

At CT, lymphoma is identified as a circumscribed homogeneous mass, with moderate homogeneous contrast enhancement, that disseminates between normal structures without compressing them. The IVC and aorta can be anteriorly modified, producing the imagistic sign called "floating aorta". Necrosis and calcifications are exceptional before therapy [23]. According to literature approximately 23% of the non-Hodgkin lymphomas are heterogeneous and they cannot be differentiated from other primary retroperitoneal tumors on the basis of their enhancement characteristics alone.

Ureterohydronephrosis, with secondary urinary tract infections [24, 25] and IVC obstruction may be found [1].

Image-guided needle biopsy is a first-line investigation when facing such cases, being useful in the diagnosis of lymphoma present in the retroperitoneum, especially if we consider its simplicity, as well as its noninvasiveness, often sparing the patients from radical procedures. Lymphoma comprises a heterogeneous group of lymphoproliferative malignancies, with varying morphologic features depending on the specific subtype [26, 27]. The abnormal lymphocytes in the lymph node, bone marrow, or in extranodal sites can be small, cleaved or noncleaved, intermediate or large cells and they can have a follicular or diffuse pattern. In contrast to reactive follicular hyperplasia, lymphomas usually alter the lymph nodes architecture, the capsule being usually involved [26, 28].

However, needle biopsy encounters several limitations; in few cases, core needle biopsy offers an amount of normal tissue or tissue that has lost its normal architecture, making diagnosis impossible [28, 29].

Immunophenotypic analysis aids to determine neoplastic from reactive lymphoid infiltrates, nonlymphoid from lymphoid malignancies, and specific lymphoid neoplasms. Although bcl-2 expression differentiates reactive follicular hyperplasia from follicular lymphoma, bcl-1 expression strongly supports a diagnosis of mantle cell lymphoma. CD30 expression is also important for the identification of anaplastic large cell lymphoma and it can also be found in the majority of Hodgkin lymphomas. Lymphoma is treated with chemotherapy or radiation therapy [30]. Soft-tissue masses can persist after treatment due to fibrosis and cannot be dif¬ferentiated from viable tumors [31].

Cystic Neoplastic Masses

Retroperitoneal cysts are infrequent, these tumors rarely producing symptoms, usually these symptoms being related to the cysts location and size, as well as to their complications. Differential diagnosis should be made between retroperitoneal cysts and omental, mesenteric, splenic and enteric duplication cysts.

Serous Cystadenocarcinoma

Serous cystadenocarcinoma is a rare primary epithelial retroperitoneal tumor that develops through mucinous or serous metaplasia of retroperitoneal coelomic mesothelium, which is located along ovarian descents [32]. Other approach of expansion includes a supernumerary ovary, an enterogenous cyst and a teratoma. Serous cystadenocarcinoma is discovered in women with normal ovaries. Histopathologically, the mass is multicystic and encapsulated, full with serous fluid and extruding papillary nodules. Imaging techniques are nonspecific and display a defined cystic mass with solid intramural nodules. Increase of the wall and solid components may be found. Other important diagnostic characteristics include: an elevated carcinoembryonic antigen level in the serum andglandular epithelial cells at biopsy [32]. This type of neoplasia needs surgical excision with wide margins [33]. Other rarely primary retroperitoneal epithelial neoplasms are mucinous cystadenocarcinoma, borderline mucinous tumor and borderline serous tumor [32].

CONCLUSIONS

Retroperitoneal masses can appear from different

tissues and are a heterogeneous group, including some rare neoplasms.

Histopathology, immunohistochemistry, imaging techniques and other diagnostic exams are valuable in the evaluation of the retroperitoneal masses, particularly in staging as well as in assessing the nearby invasion. Although for now a specific diagnosis may be difficult to determine due to a lack of diagnostic information, the identification of certain characteristic features, along with clinical and demographic information, may help in narrowing the differential diagnosis.

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REVIEW ARTICLE

Article received on September 30, 2018 and accepted for publishing on January 28, 2019.

SGLT2 inhibition in patients with type 2 diabetes and cardiovascular diseases: which are the benefits?

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Abstract: *Two major trials, EMPA-REG OUTCOME trial (with Empagliflozin) and CANVAS trial (with Canagliflozin) have demonstrated the beneficial effects of SGLT2 inhibitors in patients with type 2 diabetes at risk for heart failure. SGLT2 inhibitors possess a unique pharmacological profile and have shown a significant reduction of hospitalization for heart failure in diabetic patients, immediately after treatment commencement. It seems that their effects are beneficial both in heart failure with reduced ejection fraction and heart failure with preserved ejection fraction. SGLT2 inhibitors reduce cardiovascular endpoints in patients with diabetes and high cardiovascular risk, most likely through a reduction of heart failure-related events. Empagliflozin treatment exerts beneficial effects on vascular function and central haemodynamics. Various mechanisms seem to contribute to the beneficial effects of SGLT2 inhibitors in heart failure. Taking into consideration their cardiovascular benefits and the number of ongoing trials with these drugs, there is a possibility to expand their indication to nondiabetic patients with heart failure. This review focuses on the newest insights into the beneficial effects of SGLT2 inhibitors on the cardiovascular system.*

Keywords: *SGLT2 inhibitors, type 2 diabetes, empagliflozin, canagliflozin, heart failure*

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major risk factor for cardiovascular disease (CVD) and, moreover, their association determines a higher mortality rate [1, 2]. On the other hand, heart failure represents an independent risk factor for developing T2DM [3], and a percentage varying from 20% to 40% of the patients with heart failure have also T2DM [4].

Pharmacological treatment of patients with T2DM is complex and has been continuously improved over the years. The treatment may be with oral antidiabetic agents (OAD) and/or insulin. The main oral antidiabetic agents are:

- Sulfonylureas.
- Alpha-glucosidase inhibitors.
- Thiazolidinediones.
- Glinides.
- Biguanides.
- Dipeptidyl peptidase-4 (DPP-4) inhibitors.
- GLP-1 agonists.

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• Sodium-glucose cotransporter (SGLT2) inhibitors.

ORAL ANTIDIABETIC TREATMENT

The first agent used as OAD in the treatment of T2DM was sulfonylurea that has hypoglycemic effects by stimulating the pancreatic β-cells to secrete insulin, primarily by closing the ATP-sensitive K channels from the β-cells plasma membrane inducing a series of events finalized with insulin release [5].

Alpha-glucosidase inhibitors are a class of OAD which were developed from bacteria. They are inhibiting the action of thealpha-amylase from the intestinal brush border. Their activity is independent of the pancreas and consists of delaying carbohydrate absorption in the gastrointestinal tract, obtaining the control of postprandial hyperglycaemia and, also, cardiovascular benefits [6].

Thiazolidinediones are a class of OAD formed by selective agonists of PPARγ from adipocytes, where they stimulate the fatty acid uptake and adipogenesis, especially in peripheral visceral fat (except the visceral fat). Lowering the concentration of circulating fatty acids and available lipids in the liver and muscle results in improving the patient's sensitivity to insulin. Also, they decrease the concentrations of hormones secreted by adipocytes, particularly adiponectin. Overall, they have mixed effects on circulating lipids and increase total body fat [7].

Glinides are OAD with a rapid effect onset, but short-acting duration [8], which stimulate the pancreatic release of insulin, thus lowering postprandial glycaemia.

Biguanides are OAD, metformin being the most important representative of this class. They are antihyperglycaemic agents, mainly through inhibition of hepatic glucose release and, also, in a lesser extent, through delaying the enteric absorption of glucose and stimulating the uptake of glucose at different levels – skeletal muscle, enterocytes and adipocytes [9].

Recently, new OAD classes, such as DPP-4 inhibitors, GLP-1 agonists or SGLT2 inhibitors, have been developed.

DPP-4 inhibitors act by preventing the inactivation of glucagon-like peptide 1 (GLP-1)[10] and glucose-dependent insulinotropic peptide (GIP) [11], resulting in an increased level of active GLP-1. The overall effect is the lowering of glucose levels through increased insulin secretion and reduced glucagon secretion [10].

GLP-1 agonists are grouped in long-acting and short-acting, after their effect duration. They stimulate insulin secretion and suppress glucagon secretion [12].

SGLT2 inhibitors act on the channels from the proximal convoluted tubules, where they stimulate glucose elimination through urine, by preventing its reabsorption. There is no risk of hypoglycaemia, because they act on blood glucose level and are independent of insulin's action, thus not affecting the pancreatic β-cells. Their limitation is represented by the impairment of the renal function [13].

SGLT2 inhibitors reduce glucose reabsorption in the early proximal tubule, leading to urinary glucose excretion and osmotic diuresis [14]. This class of medication has two main representatives-empagliflozin and canagliflozin, which demonstrated their beneficial effects in patients with T2DM at risk for heart failure in two major trials – EMPA-REG OUTCOME (with Empagliflozin) and CANVAS (with Canagliflozin). SGLT2 inhibitors have hypoglycaemic effects through increasing glycosuria and inhibiting renal glucose reabsorption [15]. They have also several additional beneficial effects, such as improving blood pressure [2, 13, 16–19], intrarenal haemodynamics [2] and albuminuria, reducing body weight [2].

For the management of cardiovascular risk in patients with T2DM, glycaemic control is very important, because it reduces the frequency of diabetic complications – especially microvascular ones [20] and has significant benefits in end-stage renal disease [21, 22]. Weight loss improves cardiovascular risk factors in T2DM on the short-term [23], but not on the long-term [24]. A 10 mm Hg reduction in systolic blood pressure reduces all-cause mortality, macrovascular and microvascular outcomes [25], but even a small blood pressure reduction can diminish cardiovascularrisk [26]. In consequence, it is very important to have a multifactorial approach in the management of T2DM [27].

SGLT2 inhibitors have several potential mechanisms that could explain their cardiovascular benefits, such as decreasing cardiac inflammation [16,28], decreasing cardiac oxidative stress [16,28], decreasing cardiac apoptosis [16,28], attenuating mitochondrial dysfunction [28], improving cardiac calcium and sodium ionic homeostasis [28] and hemodynamic effects, such as reduction in blood pressure [2,16,17] and intravascular volume [16,17] via osmotic diuresis [16] and natriuresis [17].

SGLT2 inhibitors posology is in tablets with rapid oral absorption [29], reaching peak plasma concentrationin 1-2 h [29, 30], and with a half–life of 10.2-13.1 hours [29,30], resulting in only one administration per day. The molecule metabolism is primarily through glucuronidation in the liver, followed by drug excretion through the urinary and faecal route. It is important to emphasize that SGLT2 inhibitors reduce the renal filtration rate (with up to 80 g per day), resulting in the necessity, for patients with an impaired renal function, of a more attentive follow-up and adjustment of doses [30]. The usual doses are canagliflozin – initially 100 mg, with a maximum of 300 mg per day, dapagliflozin – initially 5 mg, with a maximum of 10 mg per day, and empagliflozin – initially 10 mg, with a maximum of 25 mg per day [30].

EMPA-REG OUTCOME was a randomized, double-blind, placebo-controlled trial, which included 7028 patients with T2DM and established cardiovascular disease [1], who received empagliflozin once a day (10 mg or 25 mg dosage) versus placebo, compared to the usual standard of care. The study was multicentric, with 590 sites in 42 countries, and lasted until a minimum of 691 patients developed an adjudicated primary outcome - time to the first occurrence of any of the following adjudicated components of the primary composite endpoint (major adverse cardiovascular events [MACE] – CV death [including fatal stroke and fatal MI], non-fatal MI [excluding silent MI], non-fatal stroke). The trial lasted from September 2010 to April 2013 [1]. More than 99% of the included patients had established cardiovascular disease. The median observation time was 3.1 years, with a mean treatment duration of 2.6 years. As a cardiovascular outcome, a lower percentage of the primary outcome took place in the empagliflozin group (10.5%) than in the placebo group (12.1%) (HR for empagliflozin group, 0.86; 95.02%, CI, 0.74 to 0.99; $p<0.001$ for non inferiority and $p = 0.04$ for superiority); a significantly lower risk of death from cardiovascular causes (HR 0.62; 95% CI, 0.49 to 0.77; $p<0.001$), death from any cause (HR 0.68; 95% CI 0.57 to 0.82, $p<0.001$) and hospitalization for heart failure (HR 0.65; 95% CI 0.50 to 0.85; $p = 0.002$); the occurrence of myocardial infarction or stroke had no significant differences between groups [1]. Glycaemic control, estimated through glycated haemoglobin, was improved by empagliflozin at 12 weeks by −0.54 percentage points (95% CI, −0.58 to −0.49) in the 10 mg group, and by −0.60 percentage points (95% CI: −0.64 to −0.55) in the 25 mg group; at 94 weeks, the differences between empagliflozin group and the placebo group were by −0.42 percentage points (95% CI,−0.48 to −0.36) and by −0.47 percentage points (95% CI, −0.54 to −0.41), respectively; at 206 weeks, the differences between empagliflozin and placebo were by −0.24 percentage points (95% CI, −0.40 to −0.08) and by −0.36 percentage points (95% CI, −0.51 to −0.20), respectively [1]. Cardiovascular risk factors were slightly improved in the empagliflozin group: weight, waist circumference, uric acid level, systolic and diastolic blood pressure, but with small increases in both LDL and HDL cholesterol, with no increase in heart rate [1].

CANVAS consisted of two sister, multicentric, randomized, double-blinded, placebo-controlled trials, which included a total of 10,142 patients with T2DM and high cardiovascular risk [2]. Patients were administered canagliflozin versus placebo in 667 centres from 30 countries until a minimum of 688 cardiovascular events took place. CANVAS trial lasted from 2009 until February 2017 [2]. The inclusion criteria were: patients with T2DM, age ≥ 30 years, with a history of symptomatic atherosclerotic vascular disease, or ≥ 50 years old with 2 or more risk factors for cardiovascular disease. The primary end-point was represented by time to the first occurrence of non-fatal myocardial infarction, non-fatal stroke or cardiovascular-related death. Its results consisted of fewer primary outcome events in the canagliflozin

group versus the placebo group (26.9 vs 31.5 participants, with an event per 1000 patient-years (HR 0.86; 95% CI of 0.75 to 0.97; p<0.001 for noninferiority; p = 0.02 for superiority). The renal outcome was a lesser progression of albuminuria in the canagliflozin group versus placebo group (89.4 vs. 128.7 participants, with an event per 1000 patient-years), with HR 0.73 (95% CI, 0.67 to 0.79); with a regression of albuminuria in canagliflozin group versus placebo group (293.4 vs. 187.5 participants per 1000 patient-years; HR 1.70; 95% CI, 1.51 to 1.91). An important outcome was the composite one -the 40% sustained reduction in eGFR, the need for renal replacement therapy, or death from renal causes, which was observed with a smaller frequency in the canagliflozin group versus the placebogroup (5.5 vs. 9.0 participants per 1000 patient-years HR 0.60; 95% CI, 0.47 to 0.77).

The empagliflozin patients had a mean age of 63 years, with a duration of diabetes of 10 years (57%), a mean HbA1c of 8.1%; cardiovascular risk was present in 99% of them through established cardiovascular disease, 10% of them had a history of heart failure, under treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers in 81%, statins in 77% and acetylsalicylic acid in 83% of cases [1].

The canagliflozin patients had a mean age of 63 years, with a duration of diabetes >13.5 years, with a mean HbA1c 8.2%; cardiovascular risk was present, 65% had established cardiovascular disease and 35% multiple risk factors. 14.4% had a history of heart failure and were prescribed treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers in 80%, statins in 75% and acetylsalicylic acid in 74% of cases [2].

SGLT2 inhibitors were evaluated through several trials. CANVAS-R, a prospective, randomized, double-blinded, placebo-controlled clinical trial, included patients with T2DM with a history or high risk of cardiovascular events [31]. The primary objective of this trial was limiting kidney disease progression and the results were fewer cases of albuminuria progression 430/2615 cases in the active group versus 581/2615 in the placebo group (HR 0.74), decreased cardiovascular mortality or hospitalized heart failure (61/2906 patients in active group versus 93/2905 patients in placebo group) (HR 0.66) and decreased cardiovascular death (40/2906 patients in the active group versus 60/2905 patients in the placebo group) (HR 0.68)[31]. CREDENCE trial was a randomized, double-blinded, placebo-controlled, parallel-group, multicentre study, with 4,402 participants, in whom the effect of canagliflozin on renal and cardiovascular primary composite endpoint was evaluated: end-stage kidney disease, doubling of serum creatinine, renal or cardiovascular death in subjects with T2DM and diabetic nephropathy [32, 33]. DECLARE-TIMI is a multi-national, randomized, double-blinded, placebo-controlled trial with 17,190 participants with T2DM that aimed to determine whether dapagliflozin versus placebo reduces cardiovascular events, such as cardiovascular death, myocardial infarction or ischaemic stroke [34]. Ertugliflozin CVOT is a randomized, double-blinded, placebo-controlled study, with the primary outcome the first occurrence of MACE after treatment with ertugliflozin in 8,000 patients with T2DM and established cardiovascular disease [35].

20% of diabetes mellitus patients over 65 years-old have heart failure [18]. There are very few OAD for patients with both heart failure and T2DM [18]. SGLT2 inhibitors, in this type of patients, are potentially OAD that can reduce the risk of cardiovascular mortality and heart failure [19]. They reduce all-cause mortality significantly when compared to placebo. Also, they reduce major adverse cardiovascular events when compared to placebo (OR 0.83; 95% CI 0.76–0.92; p<0.001), with the same efficacy for subgroups such as canagliflozin (OR 0.85; 95% CI 0.75–0.97; p=0.02) and empagliflozin (OR 0.82; 95% CI 0.71–0.94; p=0.006)[19]. Significant reductions were observed, when compared to placebo, for non-fatal myocardial infarction (OR 0.85; 95% CI 0.73–0.98; p=0.03) and for heart failure or hospitalisation due to heart failure (OR 0.67; 95% CI 0.59–0.76; p<0.001)(19). Insignificant reductions were observed, when compared with placebo, for non-fatal stroke (OR 1.02; 95% CI 0.85–1.21; p=0.87), atrial fibrillation (OR 0.61; 95% CI 0.31–1.19; p=0.15) and for the occurrence of unstable angina or unstable angina requiring hospitalisation (OR 0.95; 95% CI 0.73–1.25; p=0.73)[19].

CONCLUSIONS

SGLT2 inhibitors, such as empagliflozin, are very important, also, in treating patients with T2DM and heart failure, because they do not only reduce hyperglycaemia [2,13,18,19], but also lead to osmotic diuresis [18], reduce body weight [2,18,19] and blood pressure [2,13,18,19], without increases in heart rate, with favourable effects on markers of arterial stiffness and vascular resistance [18], albuminuria [18,19], and serum uric acid [13,18]. Another benefit of SGLT2 inhibitors is the reduction in the primary composite outcome that consists of cardiovascular death, non-fatal myocardial infarction or non-fatal stroke [18]. Treating heart failure in patients with T2DM is a real challenge, because of the lack of evidence-based guidelines for the management of such patients [18]. Important treatment goals are improving survival rates and preventing hospital admission [18].

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REVIEW ARTICLE

Article received on August 31, 2018 and accepted for publishing on November 26, 2018.

Efficacy and safety of Ombitasvir/Paritaprevir/Ritonavir+ Dasabuvir and Ribavirin in patients with compensated HCV cirrhosis

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Abstract: *Background and aim: Ombitasvir/Paritaprevir/Ritonavir+Dasabuvir in association with ribavirin was the only interferon-free regimen available in 2015 in Romania for compensated HCV cirrhosis. The aim of our study is to reveal our experience with interferon-free therapy in compensated HCV cirrhosis.*

Materials and methods: We conducted a multicenter prospective study including 90 patients with compensated (Child-Pugh A) HCV cirrhosis treated with Ombitasvir/Paritaprevir/Ritonavir + Dasabuvir and Ribavirin regimen for twelve weeks. The HCV infection was diagnosed using clinical, biological, and FibroMax tests. Each patient had follow-up visits at four, eight, twelve and twenty-four weeks after the initiation of the treatment.

Results: 44 (49% of) patients were female and 46 (51 % of) were male. The mean age of patients in the study was 61 years old. All the patients had HCV genotype 1b infection. 22 patients were naive and 68 had previous antiviral therapies based on pegylated IFN-α and ribavirin. At the initiation of treatment 64 (71%) of patients had cytolysis, 38 (42%) had thrombocytopenia, 19 (21%) patients had hyperbilirubinemia and 11 (12%) patients had anemia. It was noticed a decreasing of cytolysis, only 10% of 64 patients still having cytolysis after four weeks of treatment. One patient (1%) died after four weeks of treatment by complications of cirrhosis. 89 (99%) of patients had a virologic response (VR) at the end of treatment (EOT) and sustained virologic response (SVR) twelve weeks after the last dose administration.

Conclusion: The most common side effect among the patients in the study group was anemia (47% cases). Cytolysis disappeared after four weeks of treatment in 61% of patients. The interferon-free combination treatment was safe and highly effective in compensated HCV cirrhosis.

Keywords: *interferon-free regimen, HCV cirrhosis, anemia, Ombitasvir/Paritaprevir/ Ritonavir+Dasabuvir*

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INTRODUCTION

The World Health Organizations estimates that there are 150 million people infected with hepatitis C virus [1].

Hepatitis C virus (HCV) affects individuals worldwide with a high degree of morbidity and mortality. Chronic HCV can lead to cirrhosis, hepatocellular carcinoma, the need for transplantation and death. The annual incidence of hepatocarcinoma in patients with HCV

cirrhosis is between 1% to 7% [2, 3, 4, 5]. Genotype -1 is the most common HCV genotype from all the 7 genotypes [6]. Genotype-1b is the most prevalent HCV subtype in Europe, Central America, the Middle, and Asia [7].

Therapy with pegylated interferon and ribavirin for 12 weeks to 72 weeks was associated with low SVR (only 60% of them achieved sustained virologic response) and with adverse events [8, 9]. It can be a challenge for patients with cirrhosis to achieve SVR.

Sustained virologic response is defined as undetectable HCV-RNA at 12 weeks, after the end of treatment (EOT) and it is associated with improved survival and reduced risk of HCC development in patients with cirrhosis [10,11,12].

New IFN-free direct- acting antiviral (DAA) therapies have greatly improved SVR rates in patients with cirrhosis [13, 14, 15, 16, 17]. In most regimens for maximizing the SVR rates in patients with cirrhosis, it was required the addition of ribavirin [18, 19].

The available DAA are classified according to the viral target protein as NS3/4A protease inhibitor, NS5B polymerase inhibitors (including non-nucleoside and nucleoside inhibitors) and NS5A inhibitors [20].

Direct-acting antiviral agents were approved for the first time in 2011 for the treatment of hepatitis C. Although the addition of the NS3/4A protease inhibitors boceprevir, telaprevir or simeprevir to pegylated interferon and ribavirin regimens increased SVR rates in patients with genotype-1 infection efficacy rates have remained low in patients with cirrhosis [21, 22, 23, 24, 25].

The regimen containing ombitasvir/paritaprevir/ ritonavir and dasabuvir with or without ribavirin was approved by the European Medicine Agency and/or the Federal Drug Administration for the treatment of patients without and with cirrhosis Child-Pugh-Turcotte class A.

Ombitasvir (OBV) is an NS5A inhibitor co-formulated with NS3/4A protease inhibitor paritaprevir (PTV), and the pharmacokinetic enhancer ritonavir which increases peak through drug allowing for once daily PTV dosing exposure [26,27]. Dasabuvir is a non-nucleoside NS5B polymerase inhibitor. This 3-DAA regimen with or without ribavirin (RBV) is approved in many countries to treat HCV genotype-1 infection.

Most of prospective phase II and III trials included only a few patients with cirrhosis.

The regimen Ombitasvir/paritaprevir/ritonavir and dasabuvir were evaluated in two studies in patients with compensated HCV cirrhosis. One study- TURQUOISE II trial, an open-label phase III trial evaluated the 3D regimen with ribavirin for 12 weeks vs 24 weeks in patients with compensated HCV cirrhosis. The other study – TURQUOISE III phase III b, open-label study evidenced if it could be achieved a good SVR without ribavirin at 3D regimen in 60 patients with compensated HCV cirrhosis. Ombitasvir/ paritaprevir/ritonavir + dasabuvir with ribavirin became available in Romania for compensated HCV cirrhosis from November 2015 to November 2017 as the only interferon-free regimen.

We reported the results of a prospective study designed to asses the efficacy and safety of the regimen ombitasvir/paritaprevir/ritonavir+dasabuvir with ribavirin in patients with compensated HCV cirrhosis genotype-1b, with or without experienced pegIFN/RBV.[28, 29, 30].

PATIENTS AND METHODS

Study Patients

We conducted a multicenter prospective study including 90 patients with compensated (Child-Pugh A) HCV cirrhosis treated with Ombitasvir/Paritaprevir/ Ritonavir+Dasabuvir and Ribavirin regimen for 12 weeks. Patients were diagnosed by clinical, biological, and FibroMax tests.

Patients 18 years or older were eligible for the therapy if they had HCV genotype-1b infection for at least 6 months prior to screening, an HCV RNA > 15 UI/ml. Cirrhosis had to be documented by liver biopsy (Metavir score >3) or FibroMax (available within 3 months before therapy) and a Child-Pugh Turcotte score 5 or 6.

We included in the treatment-naive patients or those with prior exposure to PegIFN/RBV based therapy.

Decompensated cirrhosis and hepatocarcinoma were exclusion criteria.

Each patient had follow-up visits at four, eight, twelve and 24 weeks after treatment initiation. Biological tests included: hemoglobin, ALT, AST, platelets, total bilirubin, serum albumin, INR. We defined anemia as Hb<12 g/dl in women and Hb< 13 g/dl in men. Cytolysis was defined as values of ALT or AST > at least 2 times upper limit of normal. Hyperbilirubinemia was defined as a level of bilirubin > than 1.2 mg/dl. HCV RNA level in blood was evaluated at baseline, end of treatment (EOT) and 12 weeks after EOT. Undetectable HCV RNA was defined as a viral load <15 UI/ml, assessment by PCR. Virological response (VR) was defined as undetectable HCV RNA at EOT and sustained virologic response (SRV) as undetectable HCV RNA at 12 weeks after EOT.

Study design

All patients received the therapy for 12 weeks. Co-formulated OBV/PTV/r was administered once daily (25/150/100 mg) and DSV 250 mg twice daily. Patients were followed up at four, eight, twelve, and twenty-four weeks after treatment initiation.

Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Efficacy, safety and pharmacokinetic assessments

Plasma samples were collected at screening and at each study visit and HCV RNA levels were determined processed by real-time polymerase chain reaction using the COBAS TaqMan HCV version 2.0 (Roche Molecular Diagnostics). Efficacy was assessed by the percentage of patients with SVR12, defined as an HCV RNA below the lower limit of quantitation of 25 UI/ml and a lower limit of detection of 15 UI/ml after the last dose of study drug.

The primary efficacy endpoint was SVR12 (HCV RNA level <25 UI/ml 12 weeks after the last dose of study drug. The rates of SVR 4 weeks after the end of treatment (SVR4) and rapid virologic response (RVR; HCV RNA level <25 UI/ml) were also determined.

Treatment adverse events were collected from the start of study drug administration until 30 days after the last dose. Laboratory tests and clinical parameters were assessed during the study. The severity of the adverse events and their relationship with treatment were evaluated by the investigator.

Statistical Analysis

Number and percentage of patients in different categories are reported. Categorical variables included in the analysis were: sex, the previous treatment regimen. Continuous variables included were: age, cytolysis, thrombocytopenia, anemia, hyperbilirubinemia.

Results

Baseline Patient Demographics and Characteristics.

We screened patients from November 2015 to August 2016 at five sites in Bucharest. 90 patients were enrolled and received the study drug. The majority of patients had prior treatment experience with PegIFN/RBV. 22 patients were treatment-naive and 68 patients had experienced Peg/IFN/RBV.

Demographics and clinical characteristics are presented in Table 1 and Table 2.

Table 1. Baseline demographic characteristics

Parameter	**Patients** (n=90) n/%
Age (years)	65
Female gender	44 (49)
Male gender	46 (51)
Treatment naïve	22 (24)
Treatment experienced	58 (76)

Table 2. Baseline clinical characteristics

Parameter	**Patients** (n=90) n/%
Cytolysis	64 (71)
Anemia	11 (12)
Hyperbilirubinemia	19 (21)
Thrombocytopenia	38 (42)

Efficacy Outcomes

89-99% of patients completed 12 weeks of treatment with ombitasvir/paritaprevir/ritonavir, dasabuvir and ribavirin. After 12 weeks of treatment, 99% of patients achieved SVR 12 (89/90, 95% CI, 80%-99.9%) (Table 3). One patient died after four weeks of treatment.

Another patient (female, 60 years old, known with diabetes mellitus) gave up ribavirin after four weeks of treatment because of severe anemia (level of hemoglobin was 6 g/dl). We hadn't any virologic failure.

Table 3. Efficacy and safety of DAA regimen

Parameter	**Patients** (n=90) n/%
SVR	89 (99)
Anemia	42 (47)
Death	1 (1)

Improvement in laboratory assessments

At baseline 71 % (64) of patients had cytolysis and 42 % (38) had thrombocytopenia (Table 4).

Only 10% (9) of patients had cytolysis at the end of treatment. Thrombocytopenia was improved at the end of treatment, thus it was seen at only 26% of patients.

Table 4. Characteristics of patients at the end of treatment

Parameter	**Patients** (n=90) n/%
Cytolysis	9 (10)
Anemia	2 (47)
Hyperbilirubinemia	6 (40)
Thrombocytopenia	3 (26)

Safety outcomes

Most of the patients had adverse events and one patient died after four weeks of treatment. Anemia was the most frequent adverse event. At baseline only 12% of patients had anemia, but at the end of treatment the number of patients who developed anemia was increasing up to 47%. Another laboratory abnormality was hyperbilirubinemia. At the beginning of the treatment, only 21% of patients presented a high level of bilirubin. At the end of treatment, hyperbilirubinemia was seen in 40% of patients. One patient developed severe anemia (hemoglobin level was 6 g/dl) after four weeks of treatment and thus ribavirin was stopped.

DISCUSSION

Patients with HCV infection and cirrhosis are at the highest risk for hepatocarcinoma, decompensation and, mortality. In this study treatment with OBV/PTV/r, DSV and RBV had the SVR 12 rate of 99% in HCV genotype-1b infected patients with compensated cirrhosis. Treatment was well tolerated with a low rate of severe AE.

The most side effect among the patients in the study group was anemia in 47% cases. Cytolysis disappeared after four weeks of treatment at 61 % of patients. 89 of 99 patients (99%) had sustained virologic response at twelve weeks. One patient died after four weeks of treatment (1%). The treatment was safe and effective in compensated HCV cirrhosis.

In conclusion the 12 week 3-DAA regimen of OBV/PTV/r, DSV and RBV achieved the SVR 12 rate of 99% experienced and naive treatment patients with compensated cirrhosis HCV genotype-1b.

Acknowledgements

A part of the results of this study was presented as a poster at Digestive Disease Week 2017.

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ORIGINAL ARTICLE

Article received on November 30, 2018 and accepted for publishing on February 2 2019.

Renal involvement in primary retroperitoneal tumors

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Abstract: *Retroperitoneal tumors grow quietly and are generally large in size, more than half of them being larger than 20 cm at the time of diagnosis. They usually present several therapeutic challenges because of their rarity, relative late presentation and anatomical location, often in close relationship with several important structures in the retroperitoneal space. Due to intimate relationships with vital organs in retroperitoneum, extensive surgery with en bloc resection of the involved organ is often required, most frequently the kidneys, followed by colon, pancreas and spleen. Malignant tumors have a poor prognosis, the most significant factors regarding survival rates being delayed diagnosis, high histological grade, inoperability due to invasion into vital organs, and a positive surgical border.*

Keywords: *primary retroperitoneal tumors, renal involvement, therapeutic management*

INTRODUCTION

The retroperitoneum is one of the largest spaces in the body, located behind the abdominal cavity, between the posterior leaf of the parietal peritoneum and the lumbar muscles fascia. It is delimited superiorly by the diaphragm and extending inferiorly to the pelvic floor, reaching the external borders of the lumbar muscles laterally [1]. It contains connective tissue, kidneys, adrenals, ureter, aorta with its branches, inferior vena cava with its tributaries, a portion of the duoden and pancreas, a part of the descending and ascending colon and lymph nodes. At the kidneys level, the retroperitoneum is divided into three spaces by the anterior and posterior renal fascia, as follows: anterior pararenal space, perirenal space and posterior pararenal space [2]. Primary retroperitoneal tumors represent a rare, but interesting group of neoplasms. Even though they are located in the same space, tumors of the kidneys, adrenals and pancreas, tumors originating from the organs situated partially in the retroperitoneum and retroperitoneum metastasis do not fall into this category. Primary retroperitoneal tumors do not originate from any retroperitoneal organ (parenchymatous or not), but develop from the actual retroperitoneal tissue (lymphatic, nervous, vascular, support muscle, connective, or fibroareolar tissue) or from embryonic rests of the urogenital ridge (wolffian or müllerian ducts, germ cells, primitive notochord) [3-6].

The retroperitoneum can host a wide spectrum of pathologies. Primary retroperitoneal tumors are a rare entity, having a reported incidence of 0.3-3.0%. More than half of the retroperitoneal masses are malignant (70-85%), leaving a percentage of 15-25% of the tumors to be benign. The majority of the tumors are of mesenchymal origin (75%), followed by the neural origin (24%), and last from embryonic

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rests (less than 1%) [7].

The retroperitoneum represents the second most common site of origin of malignant mesenchymal tumours after the lower extremities [7, 8]. Liposarcomas (70%) and leiomyosarcomas (15%) are the two most frequent histological subtypes of sarcomas. These tumors present as hard abdominal masses with an irregular surface; they are surrounded by a capsule that is rapidly outgrown by tumor growth and infiltrate the posterior parietal peritoneum and the intra-abdominal viscera attached to it, thus becoming intraperitoneal by direct, and not metastatic, invasion.

Benign tumors are often an incidental finding during an investigation for unrelated symptoms. The most common primary benign pathologies encountered in the retroperitoneum include benign neurogenic tumours (schwannomas, neurofibromas), paragon-gliomas (functional or non-functional), fibromatosis and retroperitoneal lipomas [9, 10].

Due to the inaccessibility of the region and since these tumours often give none or non-specific symptoms until they have reached a substantial size, they are usually large at presentation [11]. They grow silent and aggressively and usually invade the surrounding structures. In primary retroperitoneal tumors lympha-denopathy and distant metastases are a rare entity. More than half of the retroperitoneal tumors are larger than 20 cm in diameter at the time of diagnosis, the retroperitoneum space being “adaptable”.

The urologist has a crucial role in diagnosing and treating these tumors, because they are usually found in the anatomical region commonly used for surgical access in many major urological surgeries. However, in certain cases, a multidisciplinary approach should not be avoided.

Retroperitoneal tumors occurs frequently in the 5th and 6th decade, being found more often in female than in male patients [12, 13]. Certain histological types, such as embryonal rhabdomyosarcoma, terato-ma and neuroblastoma, are commonly found in younger patients. Patients with primary retro-peritoneal masses usually present with abdominal swelling and pain, early satiety, abdominal discomfort, most having a palpable abdominal mass. Many benign tumors are discovered as an incidental finding during imaging for unrelated symptoms [14, 15]. Pain can vary in intensity and severity and can also be located in the lumbar, inguinal or gluteal region. General symptoms such as asthenia, anorexia, weight loss or prolonged fever may be present. A study found out that out of the total number of patients, 44% had on admission gastro-intestinal symptoms such as: vomiting, abdo-minal distention, bloating, constipation, dyspepsia, gastrointestinal bleeding or intestinal blockage. Neurological symptoms (lower limb weakness, sensory loss of the obturator, femoral cutaneous or sciatic nerve, radiculitis, sciatica and sphincter dysfunctions) were also found in 3 out of the total 27 patients, and 10% showed signs of venous compression (edema, varicocele, ascites and genital swelling).

Retroperitoneal masses are usually diagnosed by clinical examination associated with blood tests and imaging studies (CT, MRI, abdominal ultrasono-graphy). The imaging test of choice in the evaluation and staging of primary retroperitoneal masses is contrast-enhanced computed tomography of the thorax, abdominal and pelvic regions. It can distinguish between different densities which suggest the nature of the mass, defines the shape and size of the tumor, can discover enlarged lymph nodes and can assess the involvement of the neighboring organs [16, 17]. It can also be used to perform CT-guided biopsies. It can detect the presence or absence of pulmonary, bone, hepatic or peritoneal metastases and local or regional recurrence in patients who have already been treated [18].

It is important to confirm that the tumor is located within the retroperitoneal space and to exclude the possibility that the tumor originates from a retroperitoneal organ. Anterior displacement of the retroperitoneal organs is a strong indicator that the mass has a retroperitoneal origin. The migration of the large vessels can be helpful as well [19, 20].

Ultrasound is a fast, easy to use, repeatable method. It can reveal whether the mass is cystic or not, it can determine its volume, topography and can assess the condition of the inferior vena cava. The main

disadvantage is that it is a subjective method, dependent on the doctor's experience [19].

If after the results of the imaging studies the diagnosis remains uncertain, an exploratory laparotomy or laparoscopy remains a last diagnostic resource.

THERAPEUTIC MANAGEMENT

Complete surgical resection is the treatment of choice for primary retroperitoneal tumors, but local recurrence occurs in the majority of the patients, being responsible for more than 75% of sarcoma-related deaths. Complete surgical ablation is not always possible, because of its invasion in vital structures. Despite the possibility of performing large organ resections, the possibility of a residual tumor is very high.

Macroscopic excision, tumour grade, multifocality and histological subtype are the main prognostic factors that dictate local recurrence and overall survival rates [21-23]. Local recurrence rates are higher and occurs at an earlier interval in high-grade tumors compared with low-grade ones. The possibility of obtaining negative margins is remarkably lower at the time of local recurrence and each successive operation is more difficult than the last. In the presence of a positive surgical border following the resection of a malignant retroperitoneal tumor, the 5-year survival rate decreases to 28%.

The most significant factors regarding survival rates are delayed diagnosis, high histological grade, inoperability due to invasion into vital organs, and a positive surgical border. For patients with high-grade tumors, the mean life expectancy is approximately 20 months, increasing to 80 months in patients with low-grade retroperitoneal masses.

A study found that tumors larger than 10 cm generally had distant metastasis at the time of diagnosis [24]. The risk of local recurrence is more important than the risk of metastasis regarding the progression of malignant primitive retroperitoneal tumors [25].

Due to the fact that a radical surgery is needed and that the tumor is usually large in size, an access route that allows a wide exposure for procedures is required. Midline laparotomy is the acces route of choice, allowing simultaneous examination of the abdominal and pelvic viscera, enabling management of the large vascular trunks, additional visceral surgery and the incision can be extended for access in the thoracic cavity. Chevron incision is usually used to remove large tumors with the invasion of inferior vena cava and lombotomy should be used to remove small retroperitoneal masses [26].

Because of the high risk of local recurrence, a strict follow-up with CT or MRI testing is necessary every six months for 2 or 3 years [27, 28]. Since primary retropritoneal tumors have such high rates of local recurrence, adjuvant therapy is recommended.

The use of high-dose therapy, in an attempt to lower the rate of local recurrence, is limited because these tumors are often adjacent to radiosensitive structures with low radiation tolerance (such as kidneys and intestines). The doze usually used is about 60 Gy, studies found that therate of local recurrence is 67% with doses below 50 Gy but only 17% at doses equal to or higher than 50 Gy [27]. Lymphomas, rhabdo-myosarcomas, neuroblastomas and undifferentiated sarcomas seem to have good results with radiotherapy.

The use of chemotherapy in retroperitoneal tumors is controversial, having a similar response index with other therapeutic regimen and equally poor in single drug therapy or in combination [18]. The most commonly used drug, both in single drug therapy or in combination (adriamycin – dacarbazine, or cyclophos-phamide – vincristine – adriamycin – dacarbazine), is Adriamycin. This drug is also used for metastatic disease, as a palliative form of treatment [28, 29].

If a tumor is considered unresectable or the patient has distant metastases, a core needle biopsy may be indicated to confirm the diagnosis and to enable consideration of alternative therapy.

Palliative surgery (incomplete resection for unresectable tumor) should be considered for symptom control and may improve quality of life [28].

DISCUSSION

Due to the fact that kidneys and ureteres are retroperitoneal organs, they are usually involved by retroperitoneal masses, either by extrinsec compression or direct invasion. If the tumor is left unchecked, it will grow to cause secondary hidronephrosis and renal parenchymal destruction that will lead to chronic kidney disease with severe hydroelectrolytic imbalance and serious consequences. Resistant hypertension [30] with dyselectrolytemia [31, 32], secondary heart failure [33] and cardiac arrhythmias [34] may be the result of compromised renal arteries by extrinsec compression caused by the retroperitoneal masses [9].

Urological manifestations, caused by possible urinary tract involvement (displacement, compression and invasion) at any level (kidneys, ureter, and bladder) were not as frequent, only 25% of the patients presented chronic lower back pain, or acute renal and ureteral colic. Hematuria, polakiuria, dysuria or urinary retention usually occur in tumors located in the pelvic region, near the bladder [13]. Hematuria may also appear if the retroperitoneal mass invades the ureters or the pyelocaliceal system [35].

In terms of treatment, the complete surgical resection of the tumor en bloc with the involved organs is the gold standard. It can vary from segmental ureterectomy, followed by a surgical repair of the defect, to radical nephrectomy. In cases with extrinsec compression of the ureter and secondary hidronephrosis, with no signs of invasion, an internal (ureteral stent) or external (percutaneous nephrostomy) renal drainage method can be used, until the tumor can be surgically removed. More than 83% of the patients require resection of neighboring organs, most frequently the kidneys, followed by colon, pancreas and spleen, for achieving a negative margin [27].

If the ureteral defect after extensive surgical removal of the retroperitoneal mass is proximal to the ureterovesical junction and it is not large in size, a ureteroneocystostomy or a primary anastomosis of the ureter (ureteroureterostomy) is usually preferred. When ureteroureterostomy or ureteroneocystostomy cannot be performed, the psoas hitch ureteral reimplantation is the best approach. For defects in the middle third of the ureter after partial ureterectomy, the optimal approach is ureteroureterostomy, but if it cannot provide a tension free anastomosis, a Boari flap is an optimal, but complex approach. The optimal procedure for repair of an upper ureteral injury is a ureteroureterostomy, if it can be performed without tension. A nephropexy with fixation to the psoas tendon can provide a tension-free anastomosis. When a tension-free anastomosis cannot be achieved, even after kidney mobilization, autotransplantation, ileal or appendiceal interposition graft, nephrostomy or a cutaneous ureterostomy can be performed. The ureteral anastomosis following any type of primary repair is typically stented.

In advanced cases, with inoperable tumor and secondary ureterohydronephrosis, an urgent drainage of the obstructed kidney should be used [36]. This can be achieved either by internal drainage using a double J ureteral stent [17] or by using a percutaneous nephrostomy. A nephrostomy deteriorates the patient's quality of life and leads to frequent infections, blockage of tubes or bleeding. Ureteral stenting, on the other hand, can be associated with irritative bladder symptoms, blockage, sepsis and a high failure rate, which ultimately leads to a nephrostomy tube [28].

Wrona AJ et al wanted to offer a better solution than ureteral stenting and nephrostomy for kidney drainage. In their study, they presented a subcutaneous pyelovesical bypass as a solution for ureteric obstruction (detour bypass). A detour bypass is made of two coaxial tubes, a 27F porous polytetrafluoro-ethylene (PTFE) outer tubeand an inner 17F silicone tube extending beyond the PTFE, with perforations on both ends and a radio-opaque ring on the proximal end (for an accurate placement of the proximal end of the stent). The detour is placed subcutaneously, using a tunneling device, and the distal end of the tube is placed into the dome of the bladder by a small incison. They concluded that this method is a safe, minimally invasive and highly effective alternative in patients with ureteral obstruction caused by both malignant and benign

diseases and that the potential complications (infection, encrustation) may be easily and efficiently managed [37].

Andrea Montenegro et al presented a case of a 65 year-old female with a large retroperitoneal liposarcoma, in whom, due to the fact that it was very adherent to the left kidney, a complete resection of the tumor with concomitant left nephrectomy was practiced.

In a case report by Mariko Tanaka et al, they introduced the case of a 60 year old female who presented with abdominal distension. The CT revealed a large retroperitoneal tumor which proved to be a dedifferentiated liposarcoma at biopsy. Due to the fact that the tumor invaded the duodenum, head of the pancreas, right kidney, inferior vena cava and the aorta below the left renal artery, an extensive surgical removal of the tumor was performed, with en bloc resection of the right kidney, duodenum, head of the pancreas, a portion of the inferior vena cava and abdominal aorta, with concomitant vascular reconstruction [38].

In their study, Rodriguez et al found out that sarcomas, mainly liposarcomas, followed closely by leiomyosarcomas, were the most common type of primary retroperitoneal tumors. More than half of the patients included in the study presented at admission pain of varying severity and location (mainly abdominal and lumbar) and general symptoms such as asthenia, anorexia, weight loss or prolonged fever. Gastrointestinal manifestations (vomiting, abdominal distention, bloating, constipation, dyspepsia or intestinal blockage), due to compression/invasion of the gastrointestinal tract, were present in 44% of cases. Renal involvement was not as frequent, only one quarter of patients had urological symptoms, such as lower back pain, but also dysuria, polakiuria and urge [13].

Pereira et al reported the case of a 48-year-old male who presented for pain and right abdominal swelling. Clinical investigation revealed a right varicocele and imaging studies found a solitary hydronephrotic right kidney (personal history of left renal agenesis). Contrast enhanced computed tomography showed a retroperitoneal mass and a right hydronephrotic kidney. The particularity of this case was the presence of a solitary right kidney with left renal agenesis, with the possibility that the patient will become anephric after extensive surgery. Ultrasonography-guided biopsy was performed that confirmed a well-differentiated liposarcoma. Surgical excision of the retroperitoneal sarcoma was performed, sparring the right kidney, but the patient unfortunately died during chemotherapy [39-42].

Another case reported by the previous team presented with hypertension, anorexia, and weight loss (10 kg in 2 months). This patient had a solitary right kidney following a left radical nephrectomy subsequent to renal cell carcinoma. A right retroperitoneal mass was found during the study of his hypertension, which had a compressing effect on the right renal artery. Surgical excision of the mass was performed, but the patient died of local recurrence.

MRI of an 84-year-old male, who presented with complaints of pain and a palpable right lumbar mass, revealed a large retroperitoneal tumor invading the right kidney. Extensive surgical excision was made, with concomitant right nephrectomy.

Kazim et al stated that the location of intra-abdominal organs can shift in the presence of retroperitoneal tumors, due to the mass effect, with the kidneys being the most common viscera with an altered intra-abdominal location. In their series, they reported one retroperitoneal tumor, measuring 30 cm in diameter that pushed the left kidney to the right anterolateral direction. Since the left kidney was completely engulfed by the mass, the patient underwent extensive surgical excision of the mass and a left nephrectomy [43].

CONCLUSIONS

The retroperitoneum can host a wide variety of pathologies, including benign and malignant tumors. Primary retroperitoneal tumors are rare, almost all of them being malignant. Retroperitoneal masses grow silently, presenting late, usually invading neighboring structures at time of diagnosis. Contrast-enhanced computed tomography is the best way to evaluate this

kind of tumors. Complete surgical resection is the only potential curative treatment modality for retroperitoneal masses, but the characteristics of retroperitoneal tumors (size, proximity to abdominal organs and structures, proximity to large retroperitoneal vessels) make surgical treatment a real challenge for surgeons. The efficiency of pre-operative and post-operative radiotherapy and chemotherapy is still a controversial issue, complete surgical excision with negative margins remains the gold standard procedure.

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Article received on August 19, 2018 and accepted for publishing on November 9, 2018.

Digital techniques for anterior teeth form selection in complete edentulism

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Abstract: *The aim of this study was to compare two computerized digital esthetic design methods (DSD and Visagismile) in order to properly choose the appropriate form of the maxillary frontal teeth for complete bimaxillary edentulous patients and to validate the use of these applications in complete edentulism. An online questionnaire was submitted to a group of 100 persons (dental doctors, dental medicine students and persons activating in other fields); the respective persons were requested to choose the form of maxillary frontal teeth for a group of four complete bimaxillary edentulous patients, whose esthetic design had been performed by using the two computerized progams. Following the respondents' analysis of the esthetic previews elaborated for the four patients 60% of the total answers were in favour of DSD while the remaining 40% were in favour of Visagismile; these percentages are identical with the ones obtained on the basis of the answers given by dental doctors and dental medicine students. In case of the respondents activating in other fields the percentages were 66% in favour of DSD and 34% in favour of Visagismile. Based on the results of the study the two computerized methods were validated also for cases of complete edentulism but further studies to confirm this validation are still required. These two methods improve the dental doctor/patient communication and certainly provide a higher predictability of the esthetic results of prosthetic restorations not only for dentate patients but also in cases of complete edentulism.*

Keywords: *esthetic preview, complete edentulism, DSD, Visagismile*

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INTRODUCTION

Choosing the most appropriate form for maxillary frontal teeth has always represented a challenge in the prosthetic restorations of complete edentulism as doctors had available a limited number of parameters (facial profile, sex, age, personality), which were often subjective methods of analysis and, as a consequence, the esthetic results had a low degree of predictability. [1].

Modern dental medicine uses digital technologies also in the field of dental esthetics, thus ensuring that the esthetic results of the prosthetic restorations meet as many requirements as possible having, at the same time, a high degree of predictability, given the possibility to preview the results.

A considerable number of such digital applications for smile simulation have been developed, among which: DSD App, VisagiSmile, Photoshop CS6, Keynote, Planmeca Romexis Smile Design, Cerec SW 4.2, Aesthetic Digital Smile Design, Smile Designer Pro [2].

Smile planning applications adjust the digital technology to the smile design process. They can be used as multi-use conceptual tools, diagnosis and treatment plan preview instruments and can facilitate the communication with the patient and the dental technician, enhancing the predictability of the final results and allowing a thorough analysis of the facial and dental features otherwise superficially evaluated through clinical diagnosis, photographic assessment or by using models [3]. Among the esthetic software programs used at present in dentistry Visagismile and DSD are considered to be the most powerful tools in assessing and modifying the design of deficient smiles. [4].

Apart from the well-known DSD (Digital Smile Design) concept a new one, Visagismile, has been developed in the last years starting from the Hippocratic four types of temperament (sanguine, choleric, phlegmatic and melancholy) and the Jungian archetypes and symbols (basic geometric shapes and primary and secondary colours) [5]. The artist Philipp Halawell explained and developed the Visagism concept, which implies the creation of an image expressing a person's (patient's) type of personality, concept that has been integrated in the dental medicine, thus obtaining a teeth form that ensures a correlation between dental-facial harmony and the personality type [3].

The Visagism concept analyzes the relation between the facial features, temperament and patient's harmonious teeth configuration. The concept has been developed in the last years and validated for a large number of clinical cases (over 800). The software program is user-friendly, the doctor uploading one standard photography of the patient on the application, where it will be calibrated and the points composing the facial map of the patient will be selected. Based on the facial map and one brief psychological questionnaire (named "personality interview") filled in by the patient the application will establish the patient's temperament and appropriate teeth design. Afterwards the doctor can edit the dental configuration and superpose it on the patient's teeth in the photography so that the patient can preview the esthetic aspect to be obtained at the end of the treatment. All details related to the future teeth will be sent to the laboratory directly from the application in order to obtain the final prosthetic restoration [6].

We identified a large number of studies regarding the application of the esthetic software programs on dentate patients while the number of studies regarding the application of these programs on edentulous patients is quite limited. Starting from this finding we applied the two digital methods for choosing the form of the maxillary frontal teeth to complete edentulous patients.

The purpose of our work is to establish which of the two computerized methods used for choosing the form of the maxillary frontal teeth is more appropriate in case of complet edentulism.

MATERIALS AND METHODS

We performed a comparative study on a group of four patients of the Complete Denture Department of the Faculty of Dental Medicine within the University of Medicine and Pharmacy, "Carol Davila", Bucharest, Romania.

We used the following criteria for including the patients in the studied group: complete bimaxillary edentulous patients (both with and without dentures) and patients who gave their consent for participating in the studied group and for being photographed. The criteria for excluding the patients from the studied group were: dentate patients and non-cooperating patients.

For each patient a full set of photographs was taken, according to the protocols of the two software programs and all the data and photographs thus obtained were loaded and processed using Visagismile, DSD Keynote and Microsoft Excel programs. Previews of the form of the future artificial frontal teeth were obtained for each patient using both software programs; in achiving these previews, different steps were followed, as described in Table 1.

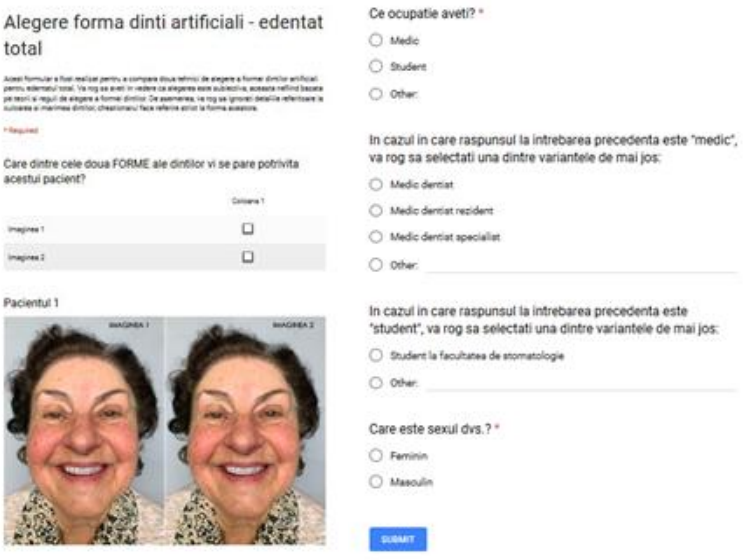
The ideal digital form of the future artificial maxillary frontal teeth was achieved for each of the four patients of the studied group by using the two computerized programs.

Table 1. Comparison between the steps for obtaining the previews corresponding to DSD Keynote and Visagismile programs

Keynote DSD	Visagismile
- Load the photograph on the software program and position the photograph in accordance to the calibrating lines - Draw the smile line and the mesio-distal limits of the future frontal teeth - Choose the forms of standard teeth that are preset by the program - Selection of the best fitting teeth-form by the doctor together with the patient - classical criteria for selection are taken into consideration (reversed face, age, sex, personality etc.)	- Load the photograph on the software program - "Auto face reading" command performs the automatic calibration - The facial map results automatically by joining 27 facial points and a "facial type" is obtained, according to the four types of temperament (choleric, sanguine, melancholy, flegmatic) - The patient is subjected to the psychological test (a short interview consisting of four questions) - The software automatically ranks the facial type in percentages depending on the statistical parameters of the facial map and the results of the interview - Patient's preferences as well as design related information can be inserted in the program in order to be submitted to the laboratory - Based on all the information inserted so far the program proposes a teeth design

In order to comparatively evaluate the two computerized methods used for choosing the form of maxillary frontal teeth with the aim of establishing which one is more appropriate for patients with complete edentulism, we submitted a questionnaire to a number of 100 persons. Out of 100 submitted questionnaires, 2 were considered invalid and 98 valid; the 98 persons were represented by 12 dental doctors, 53 students at the Faculty of Dental Medicine and 33 persons activating outside the medical field. This self-administered questionnaire (SAQ) included several questions and the two previews generated by the software programs (Figure 1), allowing us to receive feedback regarding the results obtained by using the two computerized methods from persons both activating in the dental medical field and outside the medical field. The questionnaire was filled in under the anonymity protection as the persons included in the study were not requested to provide personal information such as surname, name or personal identification number.

Figure 1. The self-administered questionnaire submitted online



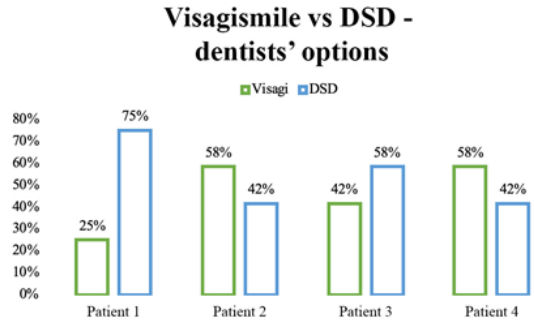
The questionnaire consisted of eight questions, of which the first four (closed-ended questions) regarded directly the four patients of the studied group while the remaining four were open-ended questions, referring to general information. The questionnaire, which was elaborated using Google Forms, was filled in online and it took sixteen days to receive all the answers. The information resulting from the answers to the questionnaires was further used, by employing Microsoft Office Excell 2018, to create statistical bases.

RESULTS

The results obtained after the questionnaire was filled in by the three categories of respondents are presented below:

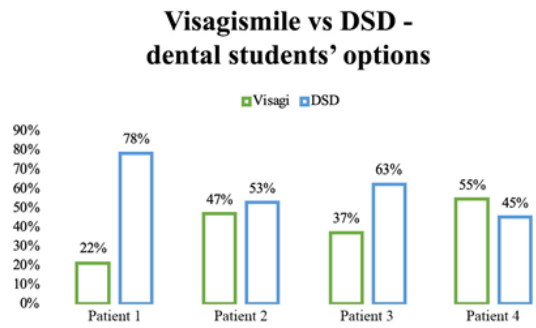
- Dental doctors opted for DSD in proportion of 60% while 40% were in favour of Visagismile (Figure 2);

Figure 2. Options expressed by dentists



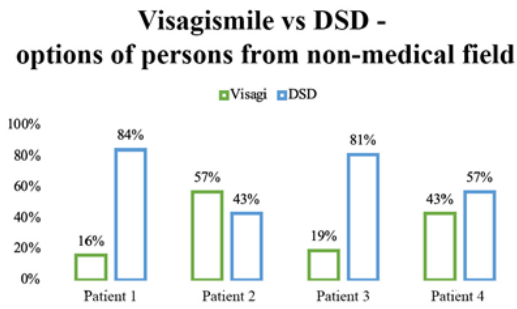
- Dental students were in favour of DSD in proportion of 60% while 40% chose Visagismile (Figure 3);

Figure 3. Options expressed by dental students



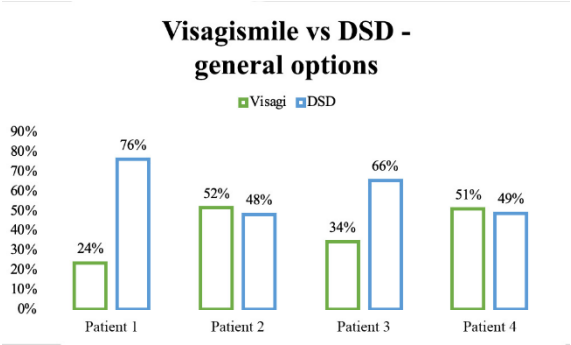
- The persons activating in non-medical fields were in favour of DSD in proportion of 66% while 34% opted for Visagismile (Figure 4).

Figure 4. Options expressed by persons from non medical field



At the level of the whole group of respondents included in the study 60% opted for DSD while 40% were in favour of Visagismile (Figure 5).

Figure 5. General options



DISCUSSIONS

There are still insufficient articles and studies illustrating the use of the smile planning digital applications to patients with complete edentulism. The modern smile design techniques are more effectively controlled through data collection and different digital smile design tools, contributing to all treatment steps up to the fabrication of the definitive restorations, either fixed or removable. Utilizing these digital tools together with classical treatment-planning rules and principles facilitates a controlled and predictable therapy when redesigning a smile [8] both for dentate patients and for complete edentulism cases.

Visagismile concept supports both dentists and

patients in deciding about the aesthetics of the final prosthetic restoration and it is mostly applied for dentate patients. Using a specific protocol, a smile design that corresponds to individual facial features, temperament and personal preferences of the patient can be achieved [7, 8]. This application can also facilitate to a great extent the conception of the complete denture design by allowing the preview of the final result and the choice of the form, colour, size and arrangement of artificial teeth depending on the facial typology and the desire of the patient, who is directly involved in elaborating the treatment plan.

In modern dentistry DSD programs are also successfully used as tools for diagnosis, treatment plan visualization and communication with the patient and the technician; all these elements can improve treatment outcome predictability. As regards the characteristics of the 3D functions, CAD/CAM connectivity and practicality, the DSD application proves to be even more performant than Visagismile. However, not all the DSD programs available today provide the same competency for the comprehensive analysis of the dentofacial esthetic parameters [8]. As in the case of complete edentulism, the actual DSD techniques can be used with certain limitations related to the following facts: dynamics of the lips when smiling; shooting of the initial photographs, which requires a good stability of the dentures; the wax occlusion rims that do not fit all the facial typologies. At the same time, the preview of the final prothestic result obtained by applying DSD offers the complete edentulous patient the possibility of having a realistic view on certain aspects related to teeth form, colour and size, which are not correlated with his/her age and facial typology.

On the other hand, the ability of the digital planning programs to perform an appropriate esthetic analysis is evaluated also depending on the number of the dental-facial esthetic parameters used. Thus, if Visagismile has the advantage of using additional elements related to the patient temperament, DSD uses more dental-facial esthetic parameters. [2] This could be the reason why the majority (between 60% and 66%) of the respondents to our questionnaire expressed their option for the DSD method.

CONCLUSIONS

Dental esthetics is a topical though subjective field and the techniques used for choosing the maxillary frontal teeth, either classical or modern (digital), have particular elements and, as a consequence, our study is a subjective one, based on the opinions expressed by the three categories of respondents.

DSD and Visagismile programs have proved their usefulness for dentate patients and our study could contribute to demonstrating their effectiveness also in case of complete bimaxillary edentulous patients. However, future studies of higher complexity and performed on larger groups of patients are necessary in order to validate the application of such programs in cases of complete edentulism.

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ORIGINAL ARTICLES

Article received on October 1, 2018 and accepted for publishing on November 14, 2018.

Renal angiomyolipoma – a short review of the literature

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Abstract: *Renal angiomyolipomas (AML) are the most common benign renal tumors. By containing an important amount of fat tissue they are easily identifiable on CT and MRI images, thus no biopsy should be needed for a diagnosis. However there is a small percentage of AMLs that have very little fat tissue and are very difficult to differentiate from renal cell carcinoma. Along fat tissue, they also contain smooth muscle and blood vessels. Renal AMLs can occur as a sporadic finding, or more frequently in association with tuberous sclerosis complex (TSC) or pulmonary lymphangioleiomyomatosis (LAM). These tumors are usually an incidental finding on imaging tools, but sometimes a serious presentation is rupture and hemorrhage, which can easily evolve to shock. Therapeutical management vary from active surveillance to surgical removal, and it needs to be individualized for every patient and according to the clinical presentation.*

Keywords: *renal angiomyolipoma, tuberous sclerosis, therapeutical management*

INTRODUCTION

Renal AMLs are the most common benign renal tumors, having a reported prevalence of 0.2-0.6%, with a female to male ratio in favor of females. Because of the increase use of imaging tools and the advances in imaging methods the frequency of detection in the general population is increasing. AMLs are more frequently associated with patients with tuberous sclerosis complex (TSC), having been reported in 75-85% of cases with renal lesions and in 49-60% in TSC patients overall [1-2]. In cases of lymphangioleiomyomatosis (LAM), aproximately 45-60% of patients tend to have renal AMLs, usually being multiple and bilateral [3-4]. Even though the prevalence of AMLs is low among patients who do not have TSC or LAM, combined with the fact that the prevalance of sporadic AML exceeds the estimated birth incidence of TSC or LAM, it makes it more common [5]. AMLs are a member of a group of tumors called neoplasms with perivascular epithelioid differentiation, arising by clonal proliferation of epithelioid cells distributed around blood vessels [6, 7].

Histological findings

Renal AMLs are made of smooth-muscle-like cells, adipocyte-like cells, and epithelioid cells, with the three types of cells appearing to be derived from pericytes, fact suggested by the fact that these cells express perycite markers [8].

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AMLs are classified in two major histological groups. The most common histological variant is the classic one, having abnormally thick-walled vessels that lack a well-developed internal elastic lamina and varying amounts of spindle smooth-muscle-like cells and adipose tissue. However, some tumors consist solely of one component, while the other elements are present in very small amounts. In the classic variant epitheliod cells are rare, making up less than 10% of cells in this type of AML [9]. Classic AMLs are bening but, there are cases with local invasion into the perirenal fat, and in very rare ocasions into the collecting system, renal vein, or inferior vena cava and right atrium. The epithelioid variant, unlike the classic one can undergo malignant transformation. There is no consensus on the percentage of epithelioid cells that is required to make a diagnosis of the epithelioid variant, but studies sugest that a percent higher than 70% of epithelioid cells, a tumor size >7 cm, vascular invasion, ≥2 mitotic figures per 10 high-power fields, atypical mitotic figures and necrosis suggest an increased risk for malignant transformation [10].

Epithelioid cells are differentiated by their abundant eosinophilic and granular cytoplasm.

Epithelioid AML has less fat tissue than the classic variant, making it more difficult to diagnose by imaging, frequently resembling and be misdiagnosed as renal cell carcinoma.

In addition to these two major histological groups, there is a rare variant, characterized by solid and cystic areas, called angiomyolipoma with epithelial cysts. It is a benign lesion with no evidence of metastasis or recurrence following surgical excision [11]. The three variants are differentiated from each other only after biopsy.

DIAGNOSIS

Clinical presentation

Commonly patients are asymptomatic at the time of diagnosis, AMLs being an incidental finding. This is explained by the increasing use of imaging tools and the advances in imaging technology [12].

Among symptomatic patients, the most common sign is flank pain, followed by a palpable mass, haematuria, anaemia, urinary tract infection, or an impaired renal function. In a study conducted by Koo et al on 129 patients, 75% of them were asymptomatic [13]. Regarding symptomatic patients, Bissler et al found that out of the 32 sypmtomatic patients included into their study 20 had flank pain, 7 had hematuria and 2 had spontaneous rupture [14].

A serious form of presentation, seen in less than 15% of cases, is spontaneous retroperitoneal haemorrhage, which can lead in one third of patients to shock. Therefore, risk of a life-threatening bleeding is the main clinical concern in a patient diagnosed with AML [12].

The epithelioid variant of AMLs can undego malignant transformation. Though rare, it is characterized by local aggressivness, lymphadenopathy, and distal metastases. The larger the tumour, the more likely it is to spread, acting like a veritable retroperitoneal tumor [15-19] and can produce secondary hydronephrosis that must be drained with a double J stent [20, 21].

AMLs tend to be more aggressive in patients with TSC or LAM, thus symptomatic patients are more commonly found in these cases. It is important to screen patients diagnosed with AML for TSC and LAM. Patients with multiple, bilateral, or larger (≥4 cm) are at higher risk for TSC and the tumors are usually diagnosed at an early age.

Imaging features

By containing fat tissue AMLs are very easily diagnosed using imaging tools. However, in cases of hemorrhage, calcification, necrosis or low fat content the diagnosis becomes a challenge. A recent article classified renal AMLs as fat-rich, fat-poor, or fat-invisible, based on the amount of fat detected on imaging studies [22].

A hyperechoic lesion, resulting from a combination of fat, blood vessel, and muscle contents, with a posterior acoustic shadow, due to the multiple tissue interfaces is the classical appearance of an AML rich in fat tissue on ultrasound [23]. The echogenity of the tumor depends on the percentage of fat tissue in the AML, usually being the same as or greater than that of

the renal sinus, but as the amount of fat gets lower, the echogenity decreases.

AML low in fat content are usually a mixture of hyperechoic and isoechoic lesions compared with the renal parenchyma, while a fat-invisible AML is homogenously isoechoic with it. In these cases it is hard to differentiate renal cell carcinoma from an AML on ultrasound [22, 24].

Computed tomography (CT) scan is recommended when hyperechoic lesion is found by ultrasound, having excellent sensitivity, specificity, and reproducibility regarding renal masses. Computed tomography can identify macroscopic fat in AML as low-density area of −10 HU or lower. Fat-poor AMLs are heterogeneously isoattenuating or hyper-attenuating, but on the other hand, fat-invisible AMLs appear homogenously hyperattenuating. Contrast-enhanced CT is not necessary for diagnosing fat-rich AML but shoud be performed where there is a risk for tumor bleeding, to identify the tortuous vessels, before therapeutic embolization can be scheduled [22, 25].

Kidney AML can be very similar with renal cell carcinomas and hemorrhagic cysts on the MRI images, thus fat suppression techniques may be helpful in differentiate them from each other [23]. As in computed tomography imaging, contrast enhanced MRI has a limited role in the diagnosis of AML.

In some cases, especially in fat-invisible AMLs, when both CT and MRI images are inconclusive and we cannot differentiate AMLs from renal cell carcinomas on imaging alone, percutaneous renal biopsy is recommended to avoid unnecessary treatment.

TREATMENT

AMLs being an incidental finding on imaging tools in asymptomatic patients, implies that a small percentage of cases requires active treatment.

The current guidelines regarding AMLs recommend active treatment in well-selected cases, such as: symptomatic tumours, the presence in women of childbearing age, poor access to follow-up or emergency care and large masses, but a size threshold remains controversial (historically a size greater than 4 cm was considered an indication for surgery).

Active surveillance

If the indication for active treatment are not present, all patients with AML should undergo routine monitoring by imaging to assess stability [26]. Patients with tumors less than 2 cm should do renal ultrasound every 3 to 4 years and those with masses measuring 2 to 4 cm should undergo ultrasound monitorisation every year. Patients with masses larger than 4 cm in diameter, who do not undergo surgery should be assesed, by ultrasound or CT every 6 months, and if stable, anually. The same with patients with epithelioid variants. Patients who have had surgical removal of an epithelioid AML, at high-risk for malignant transformation, should have whole-body CT six months after surgery and yearly thereafter for at least five years [27].

Embolization

Selective embolization of the renal artery is the preferred treatment for patients with life-threatening retroperitoneal hemorrhage. Embolization can be performed preoperatively to reduce the difficulty and complications of the surgical removal. It has a relatively high percentage of side effects including: fever, flank pain, leucocytosis, nausea, and vomiting within the first three days after the procedure, but nothing that cannot be managed conservatively. Other rare complications include non-target embolization of normal parenchyma or renal infarction [28,29]. Altough it is a generally well-tolerated procedure, it has a risk of regrowth and repeated haemorrhage after embolization [30].

Surgical excision

Partial or radical nephrectomy can be used to treat renal AMLs, being the only treatment that completely removes the renal mass. Current guidliness recommend that nephron-sparing surgery should always be performed whenever possible, being even more important in patients with TSC or LAM, because of the multifocality of the disease in this group of patients and the higher risk of recurrence. Radical nephrectomy should be used only in selected cases,

with large masses and a high risk of malignant transformation, or when other treatment options are not viable. Sometimes, in an emergency setting, a radical nephrectomy can be a life-saver [18].

Minimal invasion techniques

Cryoablation and percutaneous radiofrequency ablation can be used as an alternative to embolization or surgery. Different studies show good efficacy with minimal complications, very few therapy sessions and no recurrences, but these minimal invasion techniques should be used on small (1-3 cm in diameter) and asymptomatic lesions [31].

Medical treatment

mTOR inhibitors, such as Sirolimus or Everolimus, decreases the risk of tumor progression and reduces the size of the tumor, especially in patients with TSC or LAM., but the use of these drugs in sporadic LAMs is still debatable. Recent guidelines recommend the use of mTOR inhibitors in patients with progressive renal LAMs greater than 3 cm in asymptomatic patients with TSC or LAM as the first line of treatment. Other treatments are required in case of secondary complications, such as cardiovascular diseases [32-36] or infections [37, 38].

DISCUSSIONS

A Japanese study, using a population-based ultrasound screening, included 18,000 healthy adults, and found that 0.1 percent of men and 0.2 percent of women had renal AML [39]. Rule et al stated that renal AMLs are more common seen in middle aged women, compared to men, findings supported by Koo and his team in their research on a group of 117 patients with sporadic AML, that found that 92% of patients were women with a mean age of 52 years old [16, 40].

Ewalt et al in their longitudinal study on 60 children with TSC found that renal AMLs were present in 48% of boys and 60% of girls at a mean age of 7 years old and in 76% of boys and 83% of girls at a follow-up age of 10 years old, suggesting that the prevalence of renal AMLs in patients with TSC is increasing with age [41].

In their paper on diagnosis and management of renal angiomyolipoma, Seyam and his colleagues included 60 patients with renal AMLs with a median age of 45 years old, from which 43 were females. Half the patients presented with flank pain and only 13 had hematuria. They screened patients for TSC and found this disease in 14 patients. More than half the patients were followed up expectantly, the mean size of the tumor being 4 cm in diameter, suggesting that the majority of patients with AMLs should undergo routine monitoring to assess stability. 29 patients needed active treatment to control bleeding and relieve pain or because of the risk of malignant transformation. Tumors that are larger in size have a higher risk of bleeding, the mean size of masses in which retroperitoneal hemorrhage was discovered was approximately 11 cm. Patients were followed up for a mean of 4 years and the lesions grew an average of 5 cm for TSC patients and less than 1 cm for the sporadic group, suggesting that patients with TSC are at a higher risk of progression. Furthermore, patients whom had TSC compared with the sporadic group were younger at presentation (26 versus 49 years old), had bigger renal masses (19 cm compared with a mean of 4 cm) and had multiple or bilateral lesions [42]. Similar findings were stated in a smaller group study conducted by Nelson et al (336 patients with 19% having TSC). The patients within the sporadic renal AMLs group were older (mean 52 years old compared with 30 years old), had smaller AMLs (mean 5 compared 9 cm), and were much less likely to have both multiple lesions (13% compared with 97%) and acute hemorrhage (14% compared with 44%) [43].

Some authors recommend that renal AMLs measuring 4 to 8 cm in diameter should be observed, if asymptomatic and close follow-up can be achieved [44, 45].

Bissler and his colleagues in their paper on mTOR inhibitors included 118 patients with AMLs, 5 of which were sporadic, the rest being in correlation with TSC. The target of at least 50 percent reduction in the total volume of the mass was achieved in 42% of the patients treated with everolimus compared with none of the patients treated with placebo, with a median response time of 3 months. Progression of AMLs was significantly more common in the placebo group (21%

compared with 4% in the mTOR inhibitors treated group) [46].

mTOR inhibitors treatment can be used to reduce the volume of tumors so the patients can benefit from a nephron-sparing surgery, fact stated in Staehler's paper on „Nephron-sparing resection of angiomyolipoma after sirolimus pretreatment in patients with tuberous sclerosis". Patients treated with Sirolimus had a 38 to 95% reduction in AML volume [47].

The efficacy of selective renal artery embolization was demonstrated in a study conducted on 30 patients with large renal AMLs (mean size of 8 cm). In 83% of AMLs that were embolized, the procedure was complete after a single sesion. The median reduction volume was 43% at a follow-up of 6 months and 81% at 12 months. They found that the response was poor for fat-rich AMLs compared with fat-poor tumors [48].

De Luca stated that sporadic AMLs smaller than 1.5 cm showed no growth over 5 year follow-up in 92% of cases [49]. Furthermore, Oesterling et al said that AMLs greater than 4 cm in diameter grow more rapidly and other authors found that younger patients compared with older individuals had a higher risk of progression [50, 51]. On the other hand, epithelioid variants of AMLs have a poorer prognosis. In one study conducted on 40 patients with renal epithelioid AMLs and atypia detected on histologic examination, local recurrence or distant metastases were present in 26% of cases [52]. Nese et al had the same results in their study on 41 patients with pure epithelioid AML, out of which 36% had a metastatic disease at the time of presentation, and 16% developed metastatic disease during follow-up [53].

CONCLUSION

AMLs are a benign group of renal tumors, commonly associated with TSC and LAM, but can also appear sporadically in the population. The detection of AMLs is increasing because of an increase in the use of imaging and advances in imaging technology. The diagnosis of AML is generally made by imaging in asymptomatic patients, although a biopsy may be required to diagnose fat-poor lesions. In terms of therapeutical management, several treatment option are available, though a small percentage of patients require it and the treatment should be individualized for every patient. Most of the AMLs can be managed by active surveillance. Pure epithelioid variants of AMLs have a poor prognosis comapared with the clasic variant.

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Article received on November 28, 2018 and accepted for publishing on February 14, 2019.

Management of infectious complications in diabetes mellitus mellitus patients

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Abstract: *Diabetes mellitus is a serious condition, causing multiple health problems due to the disease itself, but also to the complications that these patients are predisposed to. Being a current health problem, many patients tend to disregard the doctor's recommendations concerning the necessary measures to maintain adequate and continuous control of the glycaemia levels. This, in turn, may cause complications, which include neuropathy, microangiopathy and a predisposition to infections due to weak local defence mechanisms.*

In otorhinolaryngology, the infectious pathology is one of the most often causes for presentation to the physician. Ranging from common otitis, acute pharyngitis or laryngitis and sinusitis, these different entities will cause significant discomfort for the patient, prompting the need for medical care. In patients with diabetes, the natural course of the disease is altered, with more severe symptoms, a more extended period of recovery and the predisposition for aggravated cases with the tendency to complications. Such complications may be life-threatening if we take into account the complications associated with acute sinusitis for example, which include orbital or cerebral abscesses.

Our paper aims to present the authors experience on the complete approach of patients with infectious pathology concerning the sinuses associating diabetes mellitus.

Always, we must keep in mind that patients with diabetes represent a particular class of patients that require a personalized approach. They are predisposed to a vicious circle, in which the infection causes diabetes imbalance, which in turn decreases the defense mechanism and predisposes to complications. The correct management must always include a proper control of diabetes, a complete evaluation that will determine if complications are already present and aggressive management of the infection, with regular check-ups to determine the evolution of the patient.

Keywords*: infections, diabetes mellitus, local complications*

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INTRODUCTION

Due to the high prevalence of diabetes mellitus in the general population, this group of patients has always benefited from practical guides concerning the proper management of the disease and the therapy protocol in case of imbalances or complications of the disease. The same principle applies for the complete approach of any secondary problems, such as electrolites imbalances in the normal body functions or associated infections [1, 2].

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It is accepted that patients with diabetes are predisposed to infectious complications due to the depressed local defense mechanisms, the body's incapacity to limit the infection and the high risk of unbalanced glycaemia levels [3-5]. However, studies that show the specific cellular dysfunctions that cause this problem are still limited. Some even stated that the link between diabetes and infections is difficult to establish [6], while other studies reinforce this concept [7]. However, the number of patients included remains limited and the conclusions scarce.

One fact remains unanimously accepted, and that is that the management of a patient with diabetes that associates an active infection is different and must be more thorough than that of a non-diabetic patient with the same illness. The guides clearly state that the infection must be limited, while the diabetes is kept under control with normal glycaemia levels, even if the use of insulin becomes necessary. In this manner we can avoid further complications that can prove to be life-threatening.

The mechanisms by which diabetes mellitus affects the normal immune capacity of the body are still unclear. Studies so far have demonstrated an association between the presence of diabetes mellitus and a decreased function of polymorphonuclear cells and macrophages [8, 9]. These cells involved in the natural processes of chemotaxis and phagocytosis present with a decreased response, thus lowering the host's capacity of self-defence. This was demonstrated when comparing the function of these cells in patients with diabetes compared to non-diabetic subjects [10].

Another mechanism that was described in order to explain the predisposition to infectious complications of diabetic patients is represented by disturbances in the humoral immunity, such as a low response of cytokines following stimulation or low levels of complement factor 4 [11].

In order to provide accurate and efficient management of these patients, it is essential to establish which mechanisms play the most significant role. Studies so far suggest that humoral adaptative immunity remains efficient also in patients with diabetes mellitus, as proven by their capacity to respond well to vaccines [12, 13]. As far as the cellular immunity is concerned, dysfunctions at this level have already been demonstrated, although at a lower level than in cases of HIV infections or other immunosuppressant conditions. Of this, one of the most important aspects is represented by lymphocytes dysfunctions in diabetes patients and delayed hypersensitivity reactions [14].

Diabetes mellitus may influence the onset and evolution of infectious diseases by other means as well. High levels of blood glucose create an environment in which a microorganism is more virulent and presents an increased adherence to the cells. This is especially true in patients with long-term unbalanced diabetes that present constant high levels of blood glucose.

Another problem that may occur in patients with diabetes and infections is the risk of diabetic ketoacidosis [3]. This complication may prove to be fatal in a large number of patients when it occurs, and it requires intensive care and aggressive management [15].

Due to all these factors, the management of the diabetic patient with an infection must be a multidisciplinary one. The team must include diabetes and nutrition specialist, an infectious diseases specialist, an intensive care unit doctor if the condition of the patients requires it, and specialist that treats the affected organ. Only with such a complex approach can these patients be balanced and healed in safe conditions.

Infections in diabetic patients can appear at any level and can involve primarily any organ. Starting from common cutaneous infections and up to severe ones, such as meningitis or myocarditis, these infections can appear alone or as part of a more severe condition and can lead to sepsis. The source of the primarly infection must be identified, and proper care measures must be ensured as soon as possible. These include broad-spectrum antibiotics as a first measure, followed by an antibiotic treatment according to an antibiogram after samples have been sent for testing. Other measures include anti-inflammatory drugs and the surgical drainage of any collection

MATERIALS AND METHODS

This paper aims to describe the proper management protocol used in our clinic for diabetic patients presenting with infections in the ENT field, more precisely the management of rhinosinusitis in diabetic patients.

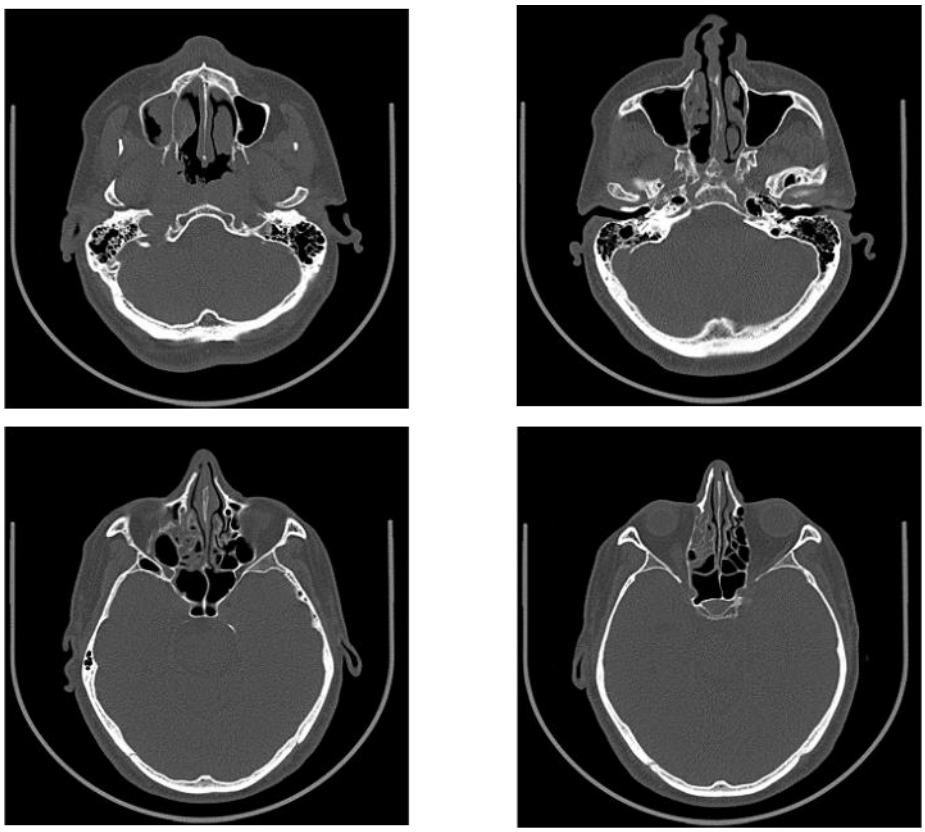
Taking into account the septic state of ENT cavities, we must keep in mind that patients with diabetes are more predisposed than the general population to develop infectious conditions. These may include otic infections, laryngeal of pharyngeal problems, but one of the most common conditions is represented by sinusitis.

Patients diagnosed with sinusitis usually associate one or more of the following complaints: purulent nasal discharge, headache, cranio-facial pressure and vertigo. In acute cases, high fever may occur. The causes of sinusitis are divided into nasal and odontogenic. The last one may represent a severe complication in patients with diabetes due to their predisposition for dental problems.

In such patients, the primary protocol is similar to that of non-diabetic patients. Antibiotic treatment associated with anti-inflammatory drugs, antihistaminics and proper hydration are the most common measures and must always associate nasal decongestants.

Complete blood work must be performed in order to asses the toll this episode inflicts on the organism. Efficient monitoring of the patient is mandatory, especially in diabetic patients, due to the risk of rapid-onset complications.

Figure 1: CT images of a patient with diabetes and sinonasal mucormycosis.



The complete diagnosis of these patients should include a computed tomography, which reveals the specific extension of any sinusitis. This is not routinely performed in the acute period, except for the cases that raise the suspicion of complications, such as an orbital abscess or meningitis. If the patients associate a positive response to therapy, the imagistic

examination should be performed at a period of time following the acute infectious episode.

Taking into account the agent that causes the infection, sinusitis can be either bacterial or fungal. Each can occur at diabetic patients, but they are at risk of developing a more significant number of fungal infections than the general population. This may be caused by both low local defense mechanisms, but also to the high incidence of dental problems in these patients.

Figure 2: Mucormycosis lesion of the mucosa of the rhinopharynx in a diabetic patient – intraoperative view.

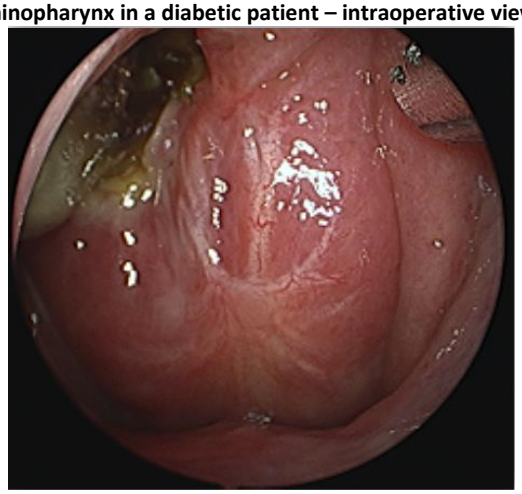


Figure 3: Intraoperative view of the same patient following an extensive debridation up to healthy tissue.

A significant aspect of the management of patients with sinusitis is the necessity to drain any purulent collection that prevents complete healing. The principle must be applied for all patients, but in diabetic patients, it is extremely important. Oral medication is more often inadequate in completely controlling the course of the disease. The surgical drainage may prove to be one of the simplest gestures that can be performed, with low risks. This principle applies to all patients but can be the only viable option in particular cases, such as Mucormycosis infections. In such cases, the surgical debridation of the affected area up to healthy tissue may be the only way to control the spread of the fungi.

The need for a multidisciplinary team remains, especially in patients with unbalanced diabetes that may require an aggressive approach [16]. Without adequate control of glycaemia levels, the healing process will be longer and less efficient. Also, an acute infection associates the risk of fluctuant glycaemia levels, with secondary complications. These patients have a higher risk of sepsis due to the decreased power of the body to contain the infection.

The surgical approach must be thorough and ensure adequate drainage of the infected sinuses. The same principle applies to bacterial or fungal sinusitis. Only the complete removal of the infectious reservoir will allow for a complete recovery.

RESULTS AND DISCUSSIONS

Patients with diabetes, especially if poorly controlled, represent a surgical challenge. Although the intervention itself follows the same principles as in non-diabetic patients, the surgeon must be even more thorough during the intervention and ensure that all infected sinuses have been drained and, in cases of fungal sinusitis, all affected tissue has been removed. The weak local defense mechanisms are not reliable in such cases, and any infectious reservoir remaining can cause a relapse in the disease [17].

These patients represent a challenge from the point of view of the anesthetist as well [18, 19]. The glycaemia levels are more difficult to control during the infectious process, they associate a higher risk of complications, including sepsis, and the response to usual therapy may be altered.

In our experience, the surgical gesture of draining the infected sinuses can be salvatory. Antibiotic or antifungal treatment is mandatory, depending on the

type of sinusitis. However, therapy alone can be insufficient and unable to limit the infection. This is even truer in diabetes patients, due to their body's low defense mechanisms. In cases of sinusitis, after a complete diagnosis that must in some cases include an imagistic evaluation, the surgical intervention is advisable if the response to treatment in the first days is insufficient or if any complications appear. The imagistic examination of choice is represented by a CT scan, which can be completed with an MRI if complications, such as orbital or cerebral extension, are suspected.

The functional endoscopic sinus surgery is a minimally invasive one and ensures a good visualization of the affected area. It allows the surgeon to control the patency of every sinus and verify if all sources of infection have been removed.

The postoperative care is crucial, being a key element in the complete recovery of the patient. Especially in a diabetic patient, maintaining appropriate glycaemia levels in the postoperative period will increase the patient's chances of a full and uneventful recovery.

CONCLUSIONS

Diabetes remains a challenging disease, despite the advancements that are continuously made concerning the therapy. The diabetic patient remains a particular one, requiring a more thorough approach, especially when an infectious process is present. People with diabetes are at higher risk to develop complications and more susceptible to inadequate and incomplete healing. Another frequent problem is caused by the multiple problems caused by diabetes, even concerning the local defense mechanisms. In such patients, a vicious circle may occur, based on the relationship between infection and unbalanced diabetes, where diabetes favors the infection. This, in turn, causes difficulties in maintaining normal glycaemia levels, which paves the way for infectious complications.

The authors recommend a multidisciplinary approach of patients with ENT infections and diabetes. This applies even more for patients with sinusitis, which is a common disease but can develop even life-threatening complications if incompletely treated. If the disease does not respond well to medical therapy, it is advisable to ensure the surgical drainage of the affected sinuses and the ENT surgeon must do so before complications. For patients with diabetes, we must also bear in mind that the onset of complications may be more rapid and dramatic.

Once the infection has been drained, the healing process will have a normal course only if the patient is well balanced, with normal glycaemia levels. Insulin may be necessary, even in patients that did not require this prior to the event. Only the proper management of diabetes allows a normal recovery. Even so, these patients must be carefully observed to ensure no secondary complications or relapses occur, and if such cases occur, the necessary measures must be quickly taken, including revision surgery if needed.

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Article received on November 28, 2018 and accepted for publishing on February 14, 2019.

Maintenance pharmacologic therapies for opioid use disorders: beyond opioid agonists

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Abstract: *Opioid use disorder is a worldwide challenge for an increasing number of patients, and still the therapeutic options presented in clinical guidelines for this condition are very few. The first line pharmacological therapies for opioid use disorder continue to be opioid agonists as substitution treatment, but the need to find new treatments without the effect of prolonging opioid dependence become more and more obvious. This systematic review presents the most supported by evidence pharmacological agents that can be used as monotherapy for opioid use disorder patients (i.e., naltrexone, extended release/implant and oral formulation), or as adjuvants to substitution therapy (e.g. olanzapine for aggressive behaviours, memantine or dextromethorphan for reducing inflammatory processes related to opioid use). More good quality data derived from larger, well designed trials are needed in order to find solid recommendations for non-opioid agonists in the treatment of opioid use disorder.*

Keywords*: opioid use disorder, naltrexone, memantine, dextromethorphan, olanzapine, maintenance treatment*

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INTRODUCTION

The importance of opioid dependence could not be overemphasized, and during the last decade a continuously increasing number of alerts have appeared both in public media and scientific environment regarding the negative functional impact, increasing societal costs and mortality risk associated with this pathology.

Opioid dependence is a significant contributor to the global disease burden, as between 24 and 35 million people globally used an illicite drug from this category in 2010 [1,2]. North America, Eastern Europe and Southern sub-Saharan Africa are the most strongly affected by the premature mortality related to opioid use disorder [1]. United Nations Office on Drugs and Crime 2017 Report shows an overall increase of the heroin and synthetic opioids use (oxycodone, hydrocodone, tramadol, and fentanyl) since 2009 and the trend to more diversification of the opioid market [3]. Disability-adjusted life years (DALY) attributable to opioid dependence in 2015 reached the value of 12 million, or 70% of the global burden of disease attributable to drug use disorder [4]. Relapse rate after detoxification is very high, ranging from 72% to 88% after 12-36 months [5].

Given consideration to the importance of opioid use disorder both at individual and societal level, worldwide efforts have been constantly made in order to find treatments able to prevent opioid use relapse and to maintain patients in remission as long as possible. The most validated treatments for the maintenance phase are methadone and buprenorphine, both with agonist actions at μ-opioid receptors (the first being a partial agonist, while the second is a full agonist). Unfortunately, due to their mechanism of action, both these drugs have abuse potential (less in case of buprenorphine) and associate withdrawal symptoms (although milder than in case of heroin or morphine). Therefore, the need to find non-addictive agents which can be used during the maintenance phase is considered urgent.

OBJECTIVE AND METHODS

This systematic literature review has as primary objective finding non-opioid agonist treatments for the maintenance phase in opioid use disorder patients, based on good quality data. The secondary objective is to formulate evidence-based recommendations for this indication, starting from existing data in the literature.

Table 1. Inclusion/exclusion criteria.

Operational criteria	Inclusion criteria	Exclusion criteria
Population	Minimum age for inclusion was 18 years, no superior age limit was established. The main diagnosis was opioid use disorder. Secondary diagnoses were allowed, if the study design permitted statistical analysis for at least one outcome regarding opioid use disorder. Diagnosis was established according to DSM-IV, IV TR, 5 or ICD-10 criteria.	Patients under age of 18. Presence of severe somatic or psychiatric co-morbidities that may have impact over patients' outcomes, or due to their treatment may lead to confusion about the outcome by pharmacokinetic or pharmacodynamic interactions.
Intervention	Any pharmacological agent without agonism (either partial or complete) over opioid receptors, either as add-on or as monotherapy. Combined pharmacological and psychological treatment. Treatment duration over 4 weeks.	Psychotherapy or psychological counselling as only type of intervention. Non-pharmacological and non-psychotherapeutical interventions, OTC, investigational products used in phase I, II or III clinical trials.
Environment	Inpatients, outpatients. Hospitals, day care, special care, intermediate care units. Carceral environment.	Unspecified environment.
Primary and secondary outcomes	Changes in opioid use- related parameters, e.g. days of abstinence, number of toxicology tests negative, rate of rehospitalisation for opioid consumption. At least one outcome is related to the opioid use.	All trials with outcomes not related to the severity of opioid use disorder.
Study design	Prospective or retrospective clinical trials. Randomized, open label, single blind, or double blind. Case control studies. Epidemiological studies. Observational trials.	Trials with unspecified or insufficiently specified design. Variables referring to opioid use disorder were not included in statistical analysis, or not presented in the published paper. Systematic review, narrative reviews, meta-analyses, case reports, case series.
Language	English, French, German, Russian	Any other language, except for those already specified.

Methods were based on the qualitative review of data found in the main electronic databases- PubMed, Complete MEDLINE, EMBASE, Cumulative Index to Nursing and Allied Health Literature (CINAHL), National Library of Medicine and the National Institute of Health (NCBI), PsychInfo and Thomson Reuters/Web of Science. All clinical trials and epidemiological studies published between 1998 and 2018 in English, French, German, and Russian were included in the primary review. The search paradigm used was „opioid dependence", „opiate dependence", „opioid/opiate use disorder", combined with „non-opioid agonists" or „non-opioid pharmacological treatment". This literature review is based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-P) principles of research [6].

Inclusion and exclusion criteria were applied to all papers found during primary search (table 1).

Therapeutic intervention was focused on drugs with non-opioid agonistic properties. If any psycho-therapeutic intervention was administered, than it was only as an add-on to the pharmacologic management, and not the main method of treatment. Because this review was focused on maintenance therapy, brief interventions were not included. The outcomes of therapeutic intervention had to be periodically evaluated, and statistically analysed. Only trial which produced inferential, statistically-based data were included in the final step of the review.

During the secondary analysis, papers were reviewed and their results were analyzed. Only papers to which full text was found were included in the final analysis.

All studies were ranked according to their level of significance (LOS). This level was determined based on the quality of the study design: (5) double-blind randomized controlled trial, with/without an active comparator/placebo; (4) single-blind trials, with/ without an active comparator/placebo; (3) open-label trials with/without an active comparator; (2) retrospective studies, observational trials; (1) any other study design.

GRADE recommendations for the level of evidence [7] were formulated based on the evidence found in the secondary analysis. GRADE recommendations included 5 levels: A= high quality= future research are very unlikely to change the validity of these recommendations; B= moderate quality= future research is likely to have an important impact on the appreciation of the recommendations; C=low quality= it is very likely that future research will have an important impact over the confidence in the estimate of effect and is likely to change the recommendations; D= very low quality= any estimate of the effect is very uncertain. Intermediate steps (+ or -) represent nuances of the recommendations based on the quality of trials and tolerability of intervention, as resulted from these trials [7].

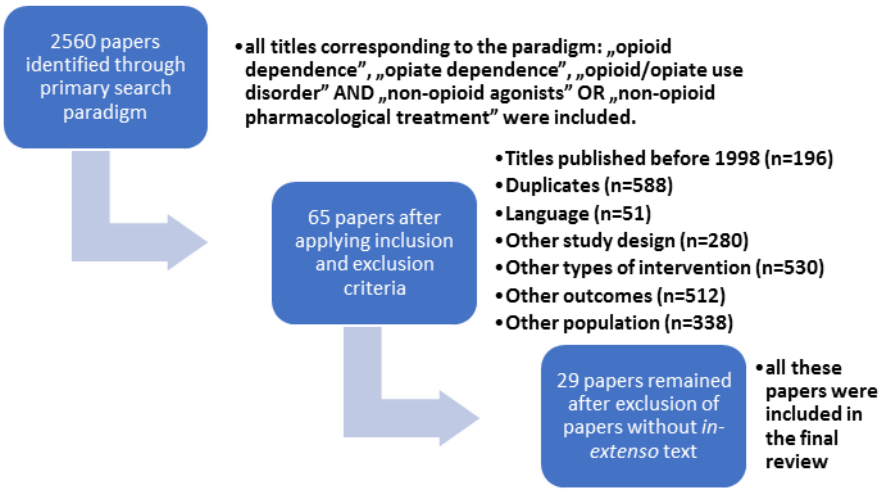
RESULTS

After the primary search 2560 papers surfaced, but only 65 remained when the inclusion and exclusion criteria were applied (figure 1). A number of papers were excluded because full text was not found, so that 29 studies remained to be reviewed in the final step. All trials included in the secondary review are presented in table 2.

These 29 trials included in the secondary analysis had a total number number of participants of 3,159, and a mean LOS of 3.58. A number of 13 trials were found to be high quality designed trials (LOS=5), meaning double-blind, either placebo-controlled or with an active comparator, randomized clinical trials.

Analysis of interventions corresponding to the inclusion/exclusion criteria previously mentioned showed that non-opioid agonist drugs used in the maintenance phase in patients with opioid use disorder who have been previously detoxified were: (1) naltrexone implant; (2) oral naltrexone; (3) topiramate; (4) memantine; (5) dextromethorphan; (6) dronabinol; (7) olanzapine; (8) baclofen; (9) ibudilast; (10) buspirone; (11) sodium valproate; (12) fluoxetine; (13) paroxetine; (14) clonazepam; (15) gabapentin. The last five drugs were used only in combination with another active medication or as active comparators, so they were not evaluated separately in this review.

Figure 1: Stepwise filtration of titles found during electronic databases search



Naltrexone was the most investigated non-opioid agonist medication for the maintenance therapy in opioid use disorder, and 15 relevant trials focus on this drug's efficacy have been found during the secondary search. Naltrexone is an opioid antagonist, and both the parent drug and its active metabolite (6β-naltrexol) are competitive antagonists of the μ-, and to a lesser extend to κ- and δ-opioid receptor (10-25 times lower affinity for κ- and δ-receptors compared to the μ-receptors) [8]. Because oral naltrexone is extensively metabolized during first-pass metabolism, and because therapeutic adherence of opioid users is known to be very low, various implant formulations of this pharmacological agent have been developed.

Oral formulation of naltrexone has been investigated in comparison with buprenorphine/naloxone in the post-detox phase [9], methadone in stabilized patients [10], buprenorphine and placebo in detoxified patients [11], methadone in opioid dependent patients who were stablized on methadone maintenance treatment for controlling depressive and anxiety symptoms [12]. Oral naltrexone as monotherapy was compared with naltrexone plus gabapentin (1200 mg/day) for the management of muscle pain and craving [13], and naltrexone plus placebo was compared with naltrexone plus sertraline for controlling depression severity and opioid craving [14]. Another trial compared naltrexone (50 mg/day) + fluoxetine (20 mg/day) with naltrexone + placebo, fluoxetine + placebo, or placebo for 6 months plus psychotherapy for all groups for the monitoring of remission rate during 6 months [15]. The mean LOS of these studies was 3.85, and an overall number of 657 patients were included.

These trials supported a moderate efficacy and tolerability of oral naltrexone: buprenorphine/naloxone was superior to naloxone in the reduction of opioid-positive urine test and preserving patients participation in the treatment [6], naltrexone induced similar weight gain during 6 months of treatment when compared to methadone [16], naltrexone may improve depression in patients with opioid use disorder at 6 months [12], and naltrexone was inferior to buprenorphine (not statistically significant) in several outcomes regarding the rate of relapse in detoxified heroin dependent patients [11].

Naltrexone plus fluoxetine and individual psychotherapy has been proven a useful combination especially in women with heroin dependence, because it increased the remission rate and maintenance of remission in this population [15]. Naltrexone plus gabapentin improved muscle pain and psychological dependence severity in patients dependent on pentazocine [13]. Naltrexone plus sertraline was a well-tolerated combination and increased on short-term treatment retention of recently non-depressed opioid abstinent patients, but the difference between this combo and naltrexone alone disappeared in time [14].

Naltrexone extended-release has been investigated in comparison with buprenorphine/naloxone [16], treatment as usual [5], oral naltrexone plus psychosocial therapy [17], and methadone [18]. Naltrexone implant was investigated also in several trials as single pharmacological agent, without an active comparator [19-22]. The mean LOS of these trials was 2.8, and the global number of participants was 1982.

The overall efficacy of naltrexone extended-release was good, although data are derived from poor and medium-quality designed trials. Its efficacy and tolerability were similar to buprenorphine/naltrexone in patients who wanted to switch from this combination of drugs to naltrexone implant [16], and it induced a lower rate of relapse when compared to treatment as usual, at least on short-term [23]. Heroin dependent patients who received extended release formulation of naltrexone had better outcomes than those treated with oral formulation of the same drug [17], and naltrexone implants but not methadone were associated with long-term benefits in decreasing opioid-related hospital morbidity [18].

In trials without an active comparator, naltrexone extended-release was efficient in preventing opioid overdose [22], improved depression severity at 4 weeks post-baseline, but it may also be responsible of worsening late insomnia [21], improved quality of life, rate of retention, opioid-negative urines, opioid craving, and rate of re-employment [20]. Also, naltrexone plus psychological counselling improved the acceptance of opioid dependence pharmacotherapy and prolonged the duration of abstinence to opioids, when compared to placebo plus counselling [19].

Topiramate is an anticonvulsant with GABA-enhancing properties and AMPA and kainate-inhibitory actions, involved in the modulation of extracellular dopamine release in the mesocortical and limbic system dopaminergic system, properties which recommended it as a potential treatment for cocaine dependence [24]. Patients with opioid use disorder who presented also cocaine dependence did not benefit from the addition of topiramate to their methadone maintenance therapy, because no difference to placebo could be found after 12 weeks [25].

Ibudilast is an anti-inflammatory drug with phosphodiesterase inhibition properties (especially PDE4) which has been associated in animal studies with the capacity of alterating several opioid-mediated effects [26, 27]. When compared with placebo in a 6-week RCT in patients detoxified from morphine, ibudilast treated patients presented lower rates of drug liking (oxycodone), lower craving to heroin/ tobacco/cocaine and lower subjective ratings of pain vs. placebo [26].

Memantine is a noncompetitive NMDA-receptor antagonist that blocks the effects of excessive concentrations of glutamate, currently used for Alzheimer Disease- related neurocognitive disorder and researched intensively for other dementias, ADHD, and as an agent able to reverse tolerance to opioids [28]. Memantine was administered in patients who were on buprenorphine/naloxone maintenance treatment, and compared to placebo [29], or in patients who were on methadone-maintenance-therapy, compared to placebo plus methadone-maintenance therapy [30, 31]. Memantine was also investigated as maintenance treatment in detoxified non-treatment seeker heroin dependent patients during 8 weeks of in-patient regimen [32].

These trials investigating the efficacy of mematine had a mean LOS of 4.25, and an overall number of 350 participants. It can be observed that the quality of trials focused on memantine efficacy was higher than previously mentioned research for other investigational products.

Memantine has been proven as a useful adjunctive medication for patients treated with buprenorphine/naloxone and it may provide an easier transition to buprenorphine-free therapeutic regimen [29]. Low dose memantine attenuated chronic opioid-dependence-induced cognitive impairment, decreased the daily dose of methadone, and had positive effect over probably NMDA-mediated inflammatory processes [30, 31]. Memantine decreased the subjective, but not the reinforcing

effects of heroin in non-treatment seeker heroin-dependent patients [32].

Dronabinol is a synthetic form of 9-tetrahydro-cannabinol, with similar affinity for cannabinoid 1 and 2 receptors, currently approved for use in anorexia associated with weight loss in AIDS and for the treatment of nausea associated with chemotherapy resistant to other anti-nausea treatments [33]. A double-blind, placebo-controlled RCT investigated the effects of dronabinol in patients with naltrexone extended release and a significant effect of this drug in decreasing the severity of opiate withdrawal has been proven [34].

Dextromethorphan is an antitussive drug with N-methyl-D-aspartate (NMDA) receptor antagonist properties, involved also in protecting monoaminergic neurons against inflammation-mediated degeneration [35, 36]. Chronic opioid administration produces systemic inflammation, and for this reason the use of a drug with anti-inflammatory properties seems to be granted [37]. Based on its neuroprotective and anti-inflammatory properties, dextromethorphan was investigated as adjunctive agent in patients with opioid use disorder. Dextromethorphan 60 mg/day and 120 mg/day were compared with placebo as adjunctive to methadone treatment in opioid-dependent patients [37, 38], and in another trial dextromethorphan 240 mg/day was compared with placebo in methadone-stabilized patients [39].

These trials with dextromethorphan had a mean LOS of 5, and a total of 268 patients.

Dextromethorphan was useful in low doses as adjuvant treatment in methadone-treated patients both at inflammatory level and clinical level [37, 38], but in another trial the difference versus placebo was not significant in the otcome defined as mean methadone consumption [39]. The quality of life was superior in patients who reeived dextromethorphan as adjuvant to methadone versus placebo plus methadone [39].

Olanzapine is an atypical antipsychotic investigated in patients with opioid dependence who were on substitution therapy, as a drug targeting the aggresive behavior [40, 41]. The mean LOS of these trials was 3.5, and the total number of participants was 268.

In the first trial, olanzapine (2.5-15 mg) was compared with sodium valproate (600-1000 mg) in patients stabilized on methadone, and olanzapine was slightly superior in controlling aggressivity but did not differed from valproate in reducing the number of positive urine samples [40]. In the second, smaller-sample size, olanzapine was compared to fluoxetine/paroxetine and clonazepam and reduced aggressive/hostile behavior in heroin addicted patients undergoing substitution therapy [41]. In this second trial, olanzapine was superior to its comparators in the direct aggressiveness, overall hostility, verbal aggressiveness, and aggressive incidents; also, 70% of the completers achieved full remission, without significant differences between groups [41].

Buspirone is a non-benzodiazepine anxiolytic which possesses 5HT1A agonistic properties, acting as full agonist at 5HT1A presynaptic receptors and partial agonist at postsynaptic 5HT1A receptors [42]. A single trial that compared buspirone with placebo in patients with anxiety undergoing methadone therapy has been identified during the literature review [43]. Buspirone had no significant efficacy over anxiety symptoms in these patients, but it decreased depression severity and slowed down the return to substance abuse [43].

Baclofen is a GABA-B receptor agonist that may be helpful in promoting abstinence from a variety of drugs [44]. A double-blind, placebo-controlled, 12-week RCT with a LOS of 3, which included 40 patients with opioid dependence, recently detoxified, found a significantly better treatment retention in the baclofen-treated patients (60 mg/day) [45]. This trial had a small sample size and a high attrition rate, which make difficult to generalize its results.

Table 2. Trials focused on non-opioid agonist therapies during the post-detoxification phase in patients with opioid use disorder

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Pirnia B, Soleimani AA, Malekanmehr P, et al. [25]	Placebo-controlled RCT; topiramate (25-300 mg/day), 12 weeks, escalating doses; patients were treated also with methadone 20-140 mg/day and brief behavioral compliance enhancement treatment for cocaine dependence.	n=50 patients with dual cocaine and opiates dependence	Weekly urine drug screens.	Topiramate was no better than placebo in reducing cocaine use, but was superior to placebo in reducing cocaine craving.	The efficacy of topiramate in cocaine use reduction in patients with dual cocaine and opiates dependence was not supported.	5
Solli KK, Latif ZE, Opheim A, et al. [16]	Prospective cohort study, naltrexone-extended release vs. buprenorphine-naloxone 3 month + participants' choice of study medication for an additional 9 months; the active drug was administered every 4 weeks (380 mg).	n=54 opioid-dependent adults continuing naltrexone-extended release and n=63 opioid dependents inducted on the same treatment	Use of heroin and other illicit substances, opioid craving, treatment satisfaction, treatment retention, adverse events, addiction-related problems	Follow-up after 9 months showed completion rates of 51.9% among participants continuing naltrexone-extended release in the follow-up and 47.6% among those inducted on the active treatment, with opioid abstinence rates of 53.7 and 44.4%, respectively.	Opioid-dependent individuals who elect to switch from buprenorphine-naltrexone after 3 months to extended release naltrexone 9 months were experiencing similar results (treatment completion and abstinence rates) and similar rate of adverse events to those who had been on extended-release naltrexone from the start.	4
Metz VE, Jones JD, Manubay J, et al. [26]	6-week RCT focused on ibudilast (a nonselective phosphodiesterase inhibitor) effects over craving, reinforcing, analgesic effects of oxycodone in human volunteers diagnosed with opioid dependence; patients received maintenance treatment with ibudilast (50 mg bid) or placebo	n=11 patients detoxified from morphine addiction	VAS for measurement of subjective effects of oxycodone.	Patients receiving active ibudilast presented decreased rates of drug liking after 15 mg of oxycodone; heroin/ tobacco/cocaine craving was reduced under active ibudilast vs. placebo; mean subjective ratings of pain were lower in the active ibudilast condition.	Ibudilast may be useful in treating opioid use disorder and may enhance the analgesic effects of oxycodone.	3

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Earley PH, Zummo J, Memisoglu A, et al. [20]	Single-arm, multisite, open-label study; extended release naltrexone injections for up to 24 months, combined with counselling via intensive outpatient substance abuse treatment programs.	n=38 enrolled opioid-dependent healthcare professionals who had been detoxified for at least 2 weeks	Opioid drug tests monthly, measurements for quality of life, opioid craving, and treatment satisfaction	At 24 months, mean opioid craving fell by 45.2% and quality of life (SF-36) -mental component score improved by 31.1% from baseline; of the 22 unemployed subjects at baseline, 45.5% improved employment status at 24 months.	Long-term extended release naltrexone was associated with better quality of life, rates of retention, opioid-negative urines, opioid-craving reduction, re-employment	3
Lee JD, Friedmann PD, Kinlock TW, et al. [23]	Open-label RCT, 24-week extended-release naltrexone vs. usual treatment (brief counseling and referrals for community treatment programs);	n=308 criminal justice offenders with history of opioid dependence, abstinent from opioids at the time of randomization	Time to an opioid-relapse event (defined as 10 or more days of opioid use in a 28-day period).	Participants assigned to extended-release naltrexone had a longer time to relapse than did those who received usual treatment, lower rate of relapse, and higher rate of opioid-negative urine samples. After one year, the rates of opioid-negative urine samples were equal. During one year, there were no overdose events in the extended-release naltrexone treated participants, while in the usual-treatment group there were 7 cases.	Extended-release naltrexone was associated with a rate of opioid relapse lower than that with usual treatment, but after one year the protective effects waned.	3
Mokri A, Chwarski MC, Taherinakhost H, Schottenfeld RS [9]	Single-site, two-parallel groups, double-blind RCT, 12-week; oral naltrexone vs. buprenorphine/ naloxone.	n=102 participants with opioid use disorder in the post-detox phase	Initial duration of opioid abstinence (urine toxicology tests).	Mean number of days of initial duration of verified abstinence was higher with buprenor-phine/naloxone (p=0.205); mean num-ber of opioid-negative urine tests was higher in the buprenorphine/ naloxone-treated subjects (p=0.049); the mean number in treat-ment was also greater in buprenorphine/naloxon e group (p=0.013); the rate of sustained, 12-week opioid abstinence was double in the buprenorphine/ naloxone group.	Sublingual buprenorphine/ naloxone was associated with a greater number of opioid-negative urine tests and treatment retention, but not significantly greater initial abstinence duration or proportions with sustained abstinence.	5

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Gonzalez G, DiGirolamo G, Romero-Gonzalez M, et al. [29]	Double-blind placebo-controlled RCT, 13 weeks; patients already received treatment with buprenorphine/naloxone 16/4 mg/day and they were randomized to memantine 15 mg or 30 mg vs. placebo.	n=80 young adults with opioid dependence	Change in the weekly mean proportion of opioid use, and cumulative abstinence rates after rapid buprenorphine discontinuation at week 9	Memantine 30 mg/day significantly improved short-term treatment with buprenorphine/ naloxone by reducing relapse and opioid use after buprenorphine discontinuation, during the last 2 weeks of study participation.	Memantine could be an useful adjunctive treatment at 30 mg daily for patients treated with buprenorphine/ naloxone and helpful in the transition to buprenorphine-free therapeutic regimen.	5
Bisaga A, Sullivan MA, Glass A, et al. [34]	Double-blind RCT, dronabinol 30 mg/day (n=40) vs. placebo (n=20); patient underwent inpatient detoxification for opioid dependence and extended-release naltrexone induction; dronabinol was administered while inpatient and 5 weeks afterwards.	n=60 patients recently de-toxified from opioids	Severity of opioid withdrawal and retention in treatment.	The severity of opioid withdrawal during inpatient phase was lower in the dronabinol group vs. placebo; rates of successful induction onto extended release naltrexone and completion of treatment were not significantly superior for dronabinol vs. placebo.	Dronabinol reduced the severity of opiate witdrawal but had no effect on rates of extended-release naltrexone treatment induction and retention.	5
Chang YH, Chen SL, Lee SY, et al. [30]	RCT, memantine plus methadone-maintenance-therapy (n_1=57) vs. placebo plus methadone-maintenance therapy (n_2=77); healthy controls were matched with methadone-treated group; 12 weeks duration of monitoring.	n=134 patients stabilized on methadone	Cognitive performances	Both treatment groups showed improvements in their cognitive performances. Memantine add-on was superior to placebo in the post-treatment cognitive improvement.	Low-dose memantine (5 mg/day) may attenuate chronic opioid-dependence-induced cognitive decline.	5
Lee SY, Chen SL, Chang YH, et al. [37,38]	12-week RCT, dextrometorphan 60 mg/day (n_1=65), 120 mg/day (n_2=65) vs. placebo (n_3=66) as adjunctive to methadone treatment in opioid-dependent patients.	n=196 opioid dependents	Methadone dose required, plasma morphine level, retention in treatment.	Patients who received 60 mg/day dextromethorphan had significantly longer treatment retention and lower plasma morphine levels than did the placebo group. Plasma TNF-alpha was significantly decreased in the 60 mg dextromethorphan group compared to placebo.	Low dose dextromethorphan is useful as concomitant therapy in methadone-treated patients both at the pathogenic-inflammatory level, and at the clinical level.	5

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Lee SY, Chen SL, Chang YH, et al. [31]	Double-blind RCT, placebo-controlled, 12-week duration; memantine (5 mg/day) (n_1=53) vs. placebo (n_2=75); patients with opioid dependence undergoing methadone maintenance therapy.	n=128 patients stabilized on methadone	Inflammatory bio-markers, overall dose of methadone	Memantine-treated patients required a somewhat lower methadone dose than did placebo-group (p=0.039), and they also had significantly lower plasma TNF-α and higher TGF-β1 levels.	Low-dose me-mantine may help patients during metha-done substitu-tion treatment because of the overall methadone dose decrease and positive impact over inflammatory process possible related to the NMDA-receptors.	5
Krupitsky E, Nunes EV, Ling W, et al. [19]	Double-blind, placebo-controlled multicentric RCT, 24 weeks; 380 mg extended-release naltrexone vs. placebo; counselling sessions were provided for both groups.	n=250 patients with opioid dependence, who underwent detoxification	Confirmed abstinence (urine drug tests and self-report).	Extended-release naltrexone-treated patients had significantly longer periods of confirmed abstinence (p=0.0002), more opioid-free days (p=0.0004), less craving (p<0.0001), more treatment-retention (p=0.0042).	Extended-release naltrexone in conjunction with psychosocial treatment improve the acceptance of opioid dependence pharmacothera py and prolongs the duration of abstinence to opioids.	5
Krupitskii EM, Zvartau EE, Tsoi-Podosenin MV, et al. [15]	RCT, patients were randomized on naltrexone (50 mg/day) + fluoxetine (20 mg/day), naltrexone + placebo, fluoxetine + placebo, or placebo for 6 months; individual psychotherapy for the maintenance of remission was applied for all gropus (one session).	n=280 patients with heroin addiction	Remission rate, maintenance of remission	Naltrexone + fluoxetine was more effective than fluoxetine + placebo (p<0.01) and placebo (p<0.001) in the remi-ssion percentage. Naltrexone was superior to placebo and fluoxe-tine in the efficacy of maintenance of remi-ssion and preventing relapse in patients with heroin addiction. The combination of naltrexone and fluoxetine was more effective compared to naltrexone monotherapy only in women.	Naltrexone and fluoxetine could be useful in patients with heroin dependence, especially in women.	5

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Zarghami M, Sheikhmoonesi F, Ala S, et al. [40]	12-week RCT, olanzapine (2.5-15 mg) vs. sodium valproate (600-1000 mg).	n=201 patients on methadone maintenance treatment	Aggressive behavior, urine toxicology.	Olanzapine and sodium valproate reduced the overt aggression and subscales of irritability, aggression, and suicidality. Olanzapine-treated patients presented more improvement in aggressivity. The percentage of positive urine samples (morphine, cannabis, methamphetamine) were not significantly different between the study groups.	Olanzapine and sodium valproate may be useful as adjunctive agent in decreasing aggressive behavior in heroin dependent individuals during methadone substitution treatment, with a slightly superior effect of olanzapine.	4
Salehi M, Zargar A, Ramezani MA [39]	Double-blind RCT, dextromethorphan vs. placebo; 6-month follow-up.	n=72 opiate abusers with methadone maintenance therapy	Methadone consumption dosage, quality of life, withdrawal symptoms.	The mean methadone consumption in the dextromethorphan-treated patients vs. control group were 62.7 mg/day vs. 70.4 mg/day. Total mean score of the quality of life was superior in the intervention group ($p>0.05$).	Dextromethorphan might be useful for opioid dependence treatment, but the differences vs. placebo were not significant.	5
Brooks AC, Comer SD, Sullivan MA, et al. [17]	Quasy-experimental comparison, long-acting injectable naltrexone ($n_1=42$) vs. oral naltrexone ($n_2=69$). Psychosocial therapy was also initiated for these patients.	n=101 patients on naltrexone	Days retained in treatment, severity of drug use	Long-acting injectable naltrexone had been associated with better outcome than oral naltrexone on days retained in treatment and other measurements for opiate use, while still other measurements for opiate use were not significantly different; heroin users with more severe baseline use showed better retention with oral naltrexone maintenance therapy, while less severe heroin users evidenced better outcomes when treated with long-acting injectable naltrexone.	Superior outcomes for heroin-dependents who received long-acting injectable naltrexone compared to oral naltrexone.	2

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Mysels DJ, Cheng WY, Nunes EV, Sullivan MA [21]	Prospective evaluation using Hamilton Depression Rating Scale in patients treated with naltrexone depot maintenance and relapse prevention therapy; 2 and 4 -week post-baseline evaluations.	n=34 patients on naltrexone	Depression severity	Significant improvement of depression scores after 4 weeks post-baseline ($p<0.0004$), somatic and cognitive-affective subscales scores significantly decline; late insomnia increased significantly at 2 weeks post-baseline.	Naltrexone induction and maintenance does not worsen depression, but it may cause sleep impairments	2
Myesels DJ, Vosburg SK, Benga I, et al. [10]	Retrospective chart review; patients were maintained on methadone (n=16) or naltrexone (n=20);	n=36 opiate dependent patients stabilized on pharmacologic al treatment	Change in body weight from baseline to 3 months and 6 months into treatment.	No difference in weight was detected at baseline, 3 or 6 months between the two treatment groups.	No statistically difference over the weight between methadone and naltrexone maintenance treatment.	2
Schottenfeld RS, Chawarski MC, Mazlan M [11]	Double-blind RCT, 24 weeks; oral naltrexone vs. buprenorphine vs. placebo	n=126 detoxified heroin-dependent patients	Days to first heroin use (urine drug tests), days to heroin relapse, maximum consecutive days of heroin abstinence, reductions in HIV risk behaviours over 6 months.	Days to first heroin use, days to heroin relapse (3 consecutive positive tests), and maximum consecutive days abstinent had all supported the superiority of buprenorphine (significant to placebo). Differences between buprenorphine and naltrexone were not significant for days to heroin relapse and maximum consecutive days abstinent. Differences between naltrexone and placebo were not significant for any outcomes. HIV risk behaviours were significantly reduced by all three treatments.	Buprenorphine was superior to naltrexone, although for the most variables monitored the differences were not statistically significant.	5
Ngo HT, Tait RJ, Hulse GK [18]	Retrospective longitudinal follow-up; methadone (n_1=522) or naltrexone implant (n_2=314) as maintenance treatment for opioid- dependent patients. Evaluations at 6 months and 3.5 years post-treatment.	n=836 patients treated for opioid dependence	Hospital admissions rates, adjusted changes in risks and rates of overdose-related and non-overdose-related hospital morbidity associated with opioid vs. nonopioid drugs.	Naltrexone implant decreased risk for overdose and non-overdose-related events at 3.5 years, but not methadone; 5 drug-related deaths after methadone and 1 after naltrexone implantation.	Naltrexone implants, but not methadone, has been associated with long-term benefits in decreasing opioid-related hospital morbidity.	2

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Gerra G, Di Petta G, D'Amore A, et al. [41]	12-week prospective, observational trial; open-label study; olanzapine vs. fluoxetine/ paroxetine+ clonazepam for controlling aggressiveness.	n=67 patients with heroin dependence undergoing substitution therapy	Hostility scores, incidence rates of aggressive incidents and attacks.	Direct aggressiveness, overall hostility scores, verbal aggressiveness scores, aggressive incidents rates were significantly lower compared to baseline in patients treated with olanzapine who comple-ted the treatment. Among completers, almost 70% achieved full substance abuse remission, 30% partial substance abuse remi-ssion, without signifycant differences between groups.	Olanzapine may be useful as adjunctive treatment in reducing aggressive/hostile behavior in heroin addicted patients who undergo maintenance substitution therapy.	3
Dean AJ, Saunders JB, Jones RT, et al. [12]	Open-label RCT, naltrexone 50 mg/day (n_1=42) vs. methadone (n_2=38) in opioid dependent patients who were stablized on methadone maintenance treatment; 6-month duration.	n=80 patients stabilized on methadone	Depressive symptoms severity, anxiety symptoms severity.	Patients who received naltrexone did not exhibit worsening of depressive symptoms; in patients attending follow-up assessments, those who received naltrexone presented an improvement in depression over time, compared to the control group; adherence to naltrexone correlated with lower depression severity scores.	Naltrexone has a positive impact over depressive symptoms during maintenance treatment in opioid dependent patients.	3
Hulse GK, Tait RJ, Comer SD, et al. [22]	Cohort study, pre-post design, data were prospectively collected; patients were evaluated before and after treatment with a sustained release naltrexone implant; a subgroup (n=146) had previously received treatment with oral naltrexone.	n=361 heroin dependent persons	Primary outcome was overdose events.	21 overdoses involving 20 persons in the 6 months pre-treatment; none was observed in the 6 months post-treatment; blood naltrexone levels at or above 3 ng/ml for approximately 6 months after injecttion; reduced number of opioid overdoses were also observed 7-12 months after implant; for those previously treated with oral naltrexone, more opioid overdoses occured in both 6-month prior to and after oral compared to the 6-month post-implant treatment.	Sustained-release naltrexone implant is efficient in preventing opioid overdose.	2

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Comer SD, Sullivan MA [32]	Detoxified, non-treatment-seeker-heroin dependent patients; 8 weeks; 0, 30, 60 mg/day memantine as maintenance treatment; intranasal heroin (0, 12.5, 50 mg); inpatient regimen.	n=8 patients with heroin dependence	Subjective ratings of drug quality, liking, willingness to pay for drugs, craving.	Memantine produced modest reductions in subjective ratings of drug quality, liking, willingness to pay for the drug, and craving for heroin. It had little influence over the reinforcing heroin effects.	Memantine could be a useful agent for decreasing the subjective but not the reinforcing effects of heroin.	2
McRae AL, Sonne SC, Brady KT, et al. [43]	RCT, 12-week, placebo-controlled study; patients on methadone who presented also anxiety symptoms; buspirone vs. placebo.	n=36 patients receiving methadone maintenance treatment	Anxiety symptoms	Buspirone did not significantly decrease anxiety symptoms in opioid-dependent patients.	Buspirone had no significant efficacy over anxiety symptoms in subjects undergoing methadone maintenance treatment, but it was associated with reduction in depression scale scores and slower return to substance use.	5
Assadi SM, Radgoodarzi R, Ahmadi-Abhari SA [45]	Double-blind, placebo-controlled, 12-week RCT; patients received fixed-schedule baclofen 60 mg/day or placebo.	n=40 patients with opioid dependence, recently detoxified	The primary outcome was retention in treatment.	Treatment retention was significantly higher in the baclofen group (p=0.01); 30% of patients treated with baclofen completed the trial vs. 10% patients on placebo. The rates of opioid-positive urine samples did not differ significantly between the two groups. Baclofen was superior to placebo in terms of opiate withdrawal syndrome and depressive symptoms. Intensity of opioid craving and self-reported opioid and alcohol use were more favourably influnced by baclofen, although not statistically significant. The drug side effects of the two groups were not significantly different.	Baclofen could be useful in the maintenance treatment of opioid dependence, but the sample size was small, and the attrition rates were high.	5

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Kumar P, Jain MK [13]	Open label trial; patients received clonidine (for detox) and later naltrexone p.o. (50 mg BD) as maintenance therapy vs. naltrexone plus gabapentin (1200 mg/day) for 3 weeks.	n=20 opioid dependent patients (pentazocine)	Muscle pain and craving (rated on visual-analogue scale), psychological dependence severity.	Patients receiving naltrexone + gabapentin scored significantly lower on both parameters- pain score and craving score ($p<0.001$), and psychological dependence was significantly lower in this group ($p<0.01$).	Gabapentin is efficient in controlling muscle aching, and no tolerance effect was observed after 3 weeks. Gabapentin may reduce pain in opioid dependent patients when added to naltrexone, signaling a possible synergistic effect. Gabapentin may help in controlling the opiate craving and potentiated the effect of naltrexone in this regard.	3
Farren CK, O'Malley S [14]	Double-blind, placebo-controlled trial, naltrexone 50 mg qd plus placebo vs. naltrexone 50 mg plus sertraline 50 mg qd; 12-week monitoring duration.	n=13 recently non-depressed abstinent opiate addicts	Depression severity, opiate craving, adverse events	Similar side effect profile in both groups, and similar retention rates. Significant negative correlation between Beck Depression Inventory scores and duration in treatment in the combined therapy group. Opiate craving decreased over time in both groups.	Naltrexone plus sertraline was well tolerated and initially successful in increasing treatment retention, but the difference to the naltrexone alone diminished in time.	4

Based on these results a number of recommendations can be made in patients who need maintenance treatment for opioid use disorder, if a drug without opioid agonistic properties is preferred (table 3). These recommendations have been hierarchized according to the GRADE system [35].

Regarding the limitations of current review it can be mentioned the overall low quality design of the clinical research, only 13 articles included double-blind, controlled, randomized trials. Another limitation is related to the low number of drugs investigated, and except for naltrexone (oral and extended-release), data regarding the efficacy or tolerability of other pharmacological agents were derived from few good quality trials. Only one reviewer was involved in the processing of data collected, so a bias related to this

aspect can not be excluded. More research is needed, and larger, well designed trials focused on a more diverse range of drugs are also expected to be launched in order to provide more accurate recommendation in a continuously extending pathology, like the opioid use disorder.

Table 3. Recommendation based on the literature review for patients with opioid use disorder with non-opioid agonist pharmacological agents

Treatment	Clinical trials	Advantages/disadvantages	GRADE recommendations
Naltrexone- oral administration	[9-15]	(+) naltrexone improve depressive symptoms in patients with opioid use disorder at 6 months (+) naltrexone plus fluoxetine and individual psychotherapy has been proven useful in women with heroin dependence (+) naltrexone plus gabapentin improved muscle pain and psychological dependence severity in patients dependent on pentazocine (+) naltrexone plus sertraline increased short-term treatment retention of recently non-depressed opioid abstinent patients (-) buprenorphine/naloxone was superior to naloxone in the reduction of opioid-positive urine test and preserving patients participation in the treatment (-) similar weight gain during 6 months of treatment when compared to methadone (-) naltrexone was inferior to buprenorphine (not statistically significant) in several outcomes regarding the rate of relapse in detoxified heroin dependent patients	B
Naltrexone- extended release	[16-23]	(+) efficacy and tolerability were similar to buprenorphine/naltrexone (+) lower rate of relapse when compared to treatment as usual, at least on short-term (+) better outcomes than those treated with oral formulation of the same drug (+) naltrexone implants but not methadone were associated with long-term benefits in decreasing opioid-related hospital morbidity (+) efficient in preventing opioid overdose (+) improved depression severity at 4 weeks post-baseline (+) improved quality of life, rate of retention, opioid-negative urines, opioid craving, rate of re-employment (+) prolonged the duration of abstinence to opioids, in combination with counselling (-) worsened late insomnia (-) requires implant of the active drug (usually subcutaneous)	B+
Topiramate	[25]	(-) negative results in patients with opioid and cocaine use disorder	D-
Ibudilast	[26]	(+) induced lower rates of drug liking (oxycodone), lower craving to heroin/tobacco/cocaine and lower subjective ratings of pain vs. placebo	C
Memantine	[29-32]	(+) may provide an easier transition to buprenorphine-free therapeutic regimen (+) induced attenuation of chronic opioid-dependence-related cognitive impairment (+) decreased the daily dose of methadone (+) it had positive effect over probably NMDA-mediated inflammatory processes in opioid users (+) it decreased the subjective effects of heroin in chronic users (-) it did not decreased the reinforcing effects of heroin in non-treatment seeker heroin-dependent patients	C+

Treatment	Clinical trials	Advantages/disadvantages	GRADE recommendations
Dronabinol	[34]	(+) it decreased the severity of opioid withdrawal (-) no effect over rates of naltrexone-treatment induction or retention	D
Dextro-methorphan	[37-39]	(+) it was useful in low doses as adjuvant treatment in methadone-treated patients both at inflammatory level and clinical level (+) the quality of life was superior in patients who received dextromethorphan as adjuvant to methadone versus placebo plus methadone (-) the difference versus placebo was not significant in the otcome defined as mean methadone consumption	C
Olanzapine	[40,41]	(+) it reduced direct aggressiveness, overall hostility, verbal aggressiveness, and aggressive incidents (+) improved the rate of treatment retention	C
Buspirone	[43]	(+) positive effect over depressive symptoms associated to methadone maintenance treatment (-) no effect over anxiety	C-
Baclofen	[45]	(+) significantly better treatment retention	C-

From the data collected in this review only extended release naltrexone seems to achieve a B+ level of recommendation, based on 8 trials, and the benefits of using extended-release naltrexone tend to overcome its potential disadvantages in patients with opioid use disorder who underwent successful disintoxication. This naltrexone formulation had positive impact over a large number of outcomes related to opioid use disorder, including rate of relapse, quality of life, opioid-related hospital morbidity, and depressive severity [16-23]. As an alternative, oral naltrexone may be tried, although negative trials exist – level B recommendation, based on 7 trials [9-15]. Naltrexone plus fluoxetine and individual psychotherapy has been proven a useful combination especially in women with heroin dependence [15]. Naltrexone plus gabapentin improved muscle pain and psychological dependence severity in patients dependent on pentazocine [13]. Naltrexone plus sertraline were well-tolerated and increased on short-term treatment retention of recently non-depressed opioid abstinent patients [14].

Memantine has a level C+ recommendation for its use as adjuvant treatment in opioid use disorder because there more data to support its efficacy are needed. Low dose memantine attenuated chronic opioid-dependence-induced cognitive impairment and decreased the daily dose of methadone [30, 31]. However, information have been gathered about its positive effect over cognition, inflammatory processes, and decrease of methadone daily dose [29-32].

Olanzapine may be recommended for controlling aggressivity in patients with opioid use disorder, as adjuvant treatment (level C) [40, 41].

Dextromethorphan was associated with some positive results, although it did not decrease the necessary daily dose of methadone when used as adjuvant agent [37-39]. This drug was however useful in improving both clinical and inflammatory outcomes, and improved patients' quality of life [37-39].

Dronabinol did not prove itself efficient in the maintenance phase of treatment, although it may be of some help in alleviating withdrawal symptoms in opioid users [34].

Data for ibudilast, baclofen, and buspirone are insufficient to allow for stronger recommendations in the targeted population, but further research may change this perspective.

CONCLUSIONS

Data showing a continued increase of illegal opioids use are overwhelming, and it became obvious that clinicians must find new solutions for treating a continuously growing population affected by opioid

use disorder. Opioid agonists like buprenorphine and methadone are considered now the first line for treatment of opioid dependent patients, but non-opioid agonist drugs should be investigated more, either as adjuvant agents or as monotherapy. These non-opioid agonist agents can lead to the administration of lower doses of opioid agonists (in case of adjuvant treatment), or even to their complete replacement, in both cases diminishing the risk for a new dependence.

From the data reviewed, extended-release naltrexone is the most supported by evidence recommendation, followed by oral naltrexone, in patients who were successfully detoxified from opioids. Naltrexone had positive results not only on the opioid use-related outcomes, but also over associated symptoms, like depression.

As adjuvant agents, olanzapine may be efficient for aggressive behaviours in opioid dependent patients, memantine could improve cognitive function and decrease the inflammatory processes associated with substance use disorders, and dextromethorphan may also help decreasing the inflammatory processes. Further research is needed in order to verify the efficacy and tolerability of olanzapine, memantine and dextromethorphan as adjuvant agents.

Disclaimer

No funding was received for this research.

The author was speaker for Servier, Eli Lilly and Bristol-Myers, and participated in clinical trials funded by Janssen Cilag, Astra Zeneca, Otsuka Pharmaceuticals, Sanofi-Aventis, Sunovion Pharmaceuticals.

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CLINICAL PRACTICE

Article received on December 17, 2018 and accepted for publishing on February 15, 2019.

Survival following repetitive ventricular fibrillation caused by monoamine oxidase inhibitor overdosing (MAOI).

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Abstract: *One of the great dreams of humanity has been the improvement of its behavioural, affective and emotional status. Depression represents the suffering of beings over their existential course, as a point of passage between the human condition and its destiny. The attempts of psychiatry to introduce efficient and increasingly performing antidepressants represent one of medicine's great achievements. At the same time, antidepressant overdosing may lead to cardiotoxic manifestations presenting lethal risks as consequences [1]. We hereby present the case of a patient that was poisoned with tianeptine, ingested for suicidal purposes, who survived repetitive episodes of ventricular fibrillation, electrically converted to sinusal rhythm. The GC/MS analytical diagnosis played an essential part in detecting tianeptine in the biological samples. The rapid and very competent therapeutic intervention allowed for the patient's survival, despite further complications. A review of main tianeptine information is also presented, as this type of intoxication may have deadly consequences.*

Keywords: *tianeptine, ventricular fibrillation, acute intoxication*

INTRODUCTION

Tianeptine (fig. 1) was discovered and patented by the French Society for Medical Research in the 1960s. It is an atypical antidepressant, used mostly to treat major depressive disorder, but also to treat anxiety, asthma and irritable bowel syndrome. Regarding its chemical structure, tianeptine has the structure of a tricyclic antidepressant (TCA), but has a pharmacological profile that is very different from other TCAs and is grouped separately from those due to its unique mechanism of action [1]. As a modulator of the serotoninergic synapse, it has good efficiency in mild/moderate forms of depression associated or not with anxiety; it does not interfere with the metabolism of other drugs at the level of P45 cytochrome. Tianeptine does not have an affinity for muscarinic, alfa1-adrenergic or type 1 histaminergic receptors, which leads to less adverse events compared to TCAs.

Initial studies have shown that following acute and repeated administrations, the presence of tianeptine led to a decrease in extracellular levels of serotonin in rats' brains without a decrease in the release of serotonine, which is why it was called a "(selective) inhibitor of serotonine reuptake".

As opposed to TCAs and selective serotonine reuptake inhibitors (SSRI), tianeptine improves the synaptic uptake function of serotonine; despite all these, its efficiency against depressive episodes is comparable with that of TCAs and fluoxetine [2, 3]. Recently (2014), it has been demonstrated

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that tianeptine is an agonist of opioid receptors (MOR). Thus, as opposed to its therapeutic effects, MOR activation is probably the one responsible for tianeptine's toxicity in high doses, those that far surpass the normal therapeutic interval [1]. The therapeutic levels for tianeptine in whole blood, according to available data, are 0.3–0.7 mg/ml.

The absorption takes place almost entirely (99% bioavailability) at GI level, it is completely metabolized by the liver and it is not affected by the first pass effect [4]. Its distribution in the organism takes place quickly, 95% of it binds to plasma proteins (especially albumine) and the mean plasmatic half-life through distribution is approximately 0.7 hours.

Table 1. Adverse events for tianeptine – classified by frequency.

Type of adverse events	Adverse events
Common (frequency >1%)	Headaches (up to 18%) Dizziness (up to 10%) Insomnia/nightmares (up to 20%) Sleepiness (up to 10%) Dry mouth (up to 20%) Constipation (up to 15%) Nausea Abdominal pain Weight gain (~ 3%) Agitation Anxiety/irritability
Less frequent (frequency 0.1-1%)	Bitter taste Flatulence Epigastralgia Blurred vision Muscular pain Premature ventricular contractions Micturition disorders Palpitations Orthostatic hypotension Tremor
Rare (frequency <0.1%)	Hepatitis Euphoria ECG modifications Pruritus-like cutaneous reactions/alergy Muscular pain

Metabolizing in the liver takes place especially through beta-oxidation and, in a lower proportion, through N-demethylation, the main metabolites being propanoic acid (inactive metabolite) and pentanoic acid (active metabolite) [5]. Tianeptine is eliminated both renally and through extrarenal ways, mostly through urine (only 8 % is eliminated in unmetabolized form), fecces (15%), bile and has a half-life of 2-3 hours. [5].

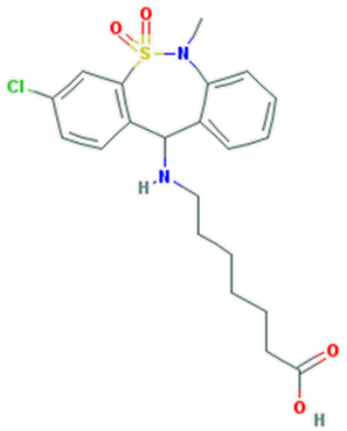
Compared to other TCAs, tianeptine can lead to significantly less cardiovascular, anticholinergic (such as constipation or dry mouth), sedative and appetite stimulating effects [6, 7]. Recent studies have shown that it is amongst the most hepatotoxic (hepatic lesions) antidepressants, although proof supporting this concern is limited [8]. Also, it has been shown that TCAs can lead to cardiac arrhythmia, even though the data for tianeptine is limited [9].

Based on adverse event frequency, they are classified according to table no. 1 [5, 6, 10].

The certitude diagnosis is established by identifying tianeptine in biological fluids.

Figure 1. Tianeptine 2D Structure form

https://pubchem.ncbi.nlm.nih.gov/compound/Tianeptine#section=Top



For this purpose, the GC/MS method (gas-chromatography coupled with mass spectrometry) is used. The GC/MS method, applied in the Analytical Toxicology Laboratory of the Military-Medicine Research Center, is performed by a GC/MS Varian SATURN 2000 computerized system: Varian gas-chromatograph STAR 3800 CX model and Varian mass spectrometer with an ion trap detector. The urine samples are analyzed using liquid-liquid extraction with organic solvents. For the extraction control, midazolam was used as an internal standard, which is

a benzodiazepine whose retention time is situated in a different range from that of tianeptine. Mass spectra libraries were used, namely Pfleger–Maurer–Weber, NIST 98 and Wiley 6.

Figure 2: Total ion chromatogram and the mass spectra obtrained for lidocaine (peak no. 1), for tianeptine (peak no. 2) and for midazolam (peak no. 3).

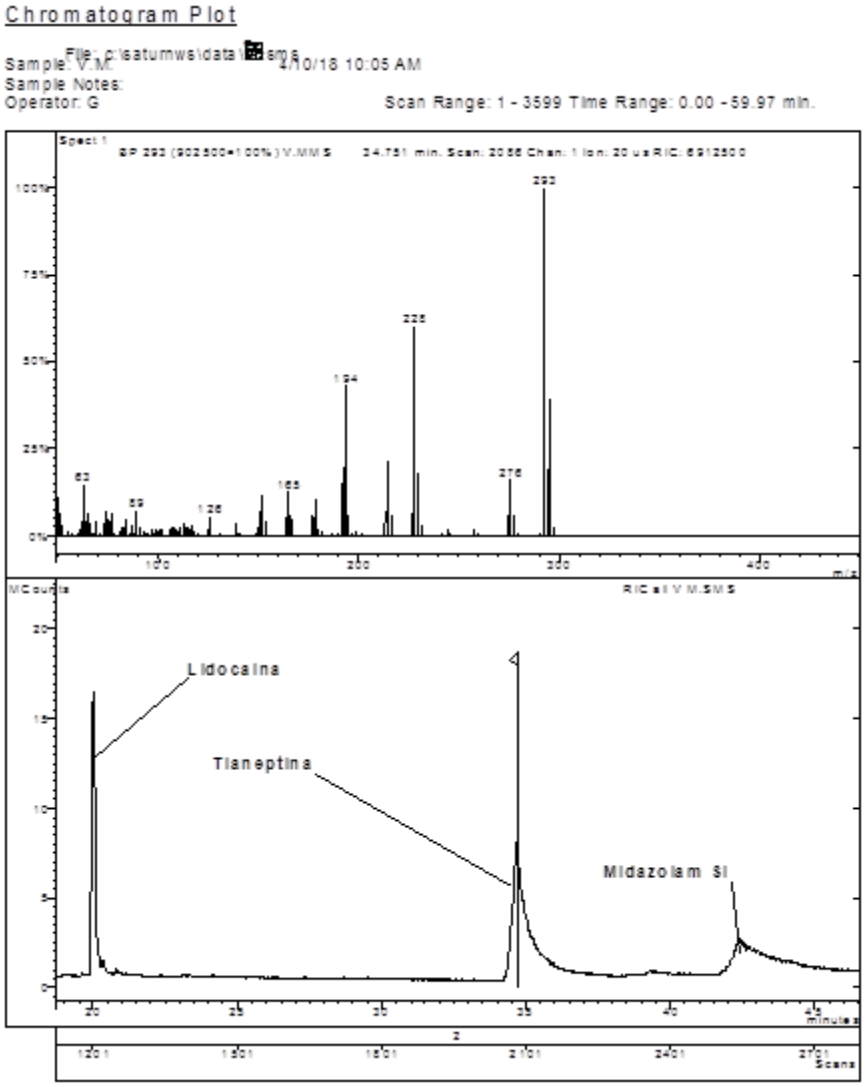


Table 2. The type of compound that was identified, its chemical formula and characteristic spectral lines

Identified compound	Chemical formula	Molecular mass	Spectral lines (m/z)
Lidocaine	$C_{14}H_{22}N_2O$	234	86, 234
Tianeptine	$C_{21}H_{25}ClN_2O_4S$	437	194, 214, 228, 276, 293
Midazolam (SI)	$C_{18}H_{13}ClFN_3$	327	310, 327

Following the analysis of the urine extract through the GC/MS method, the separation and identification of the following compounds were achieved: Lidocaine, Tianeptine and Midazolam (internal standard), presented in figure 2 and table 2.

CASE REPORT

Patient, aged 56 years old, with no pathologic history was brough by the medical ambulance to the Bagdasar hospital after being found at home following drug intake for suicidal purposes (declarated Nobixal –

Tianeptine) with agonistic respiration.

At home, the patient suffered two episodes of ventricular fibrillation, remitted after external electrical shocks (EES) applied by the ambulance medical staff. In the emergency care unit, the patient suffered another episode of ventricular fibrillation which responded to EES, with hemodynamic instability still persisting. Due to acute respiratory insufficiency, orotracheal intubation was necessarily performed.

Cardiological evaluation: arterial pressure 90/60 mmHg, AV 100 bpm, no murmurs, no rales, no edemas; ECG: regular sinusal rhythm, Q wave in DII, DIII, aVF.

The ECG shows a moderate/severe dystolic dysfunction of the left ventricle, most probably in the context of intoxication, followed by an EES, applied for rhythm disorders. The patient is sent to Emergency Clinical Hospital Bucharest, Intensive Care Unit II Toxicology.

Upon admission into Toxicology, the patient is in a serious condition sedated, orotracheal intubation with spontaneus respiration, presence of bilateral pulmonary vesicular murmur, hemodynamic instability on vasopressor support, arterial pressure = 100/58 mmHg, AV = 96/bpm, regular rhythm, normal abdominal movement, normal diuresis.

Biological parameters upon admission: increased values of glycemia, creatinine, liver enzymes (ALT and AST), LDH but also leukocytosis; coagulation tests being in normal ranges.

Table 3. Biological parameters

Sample	Result	
	Measured values	Normal values
Glycemia (*mg/dl*)	138	75-110
Urea (*mg/dl*)	25	19-43
Creatinine (*mg/dl*)	1.46	0.7-1.4
AST (*U/l*)	81	21-72
ALT (*U/l*)	107	14-50
LDH (*U/l*)	481	150-450
Sodium (*mmoles/l*)	144	137-145
Potassium (*mmoles/l*)	4.51	3.50-5.00
Leucocytes (*x 10^3/mm^3*)	16,8	4-9
Hemoglobine (*g/dl*)	13.10	12.6-17.2
Trombocytes (*x 10^3/mm^3*)	228	150-350
APTT (*sec*)	25.30	24-36.5
PT (*sec*)	13.1	11-14.5
INR	1.08	0.81-1.16

The toxicological urine analysis through the GC/MS method shows the presence of Tianeptine and Lidocaine.

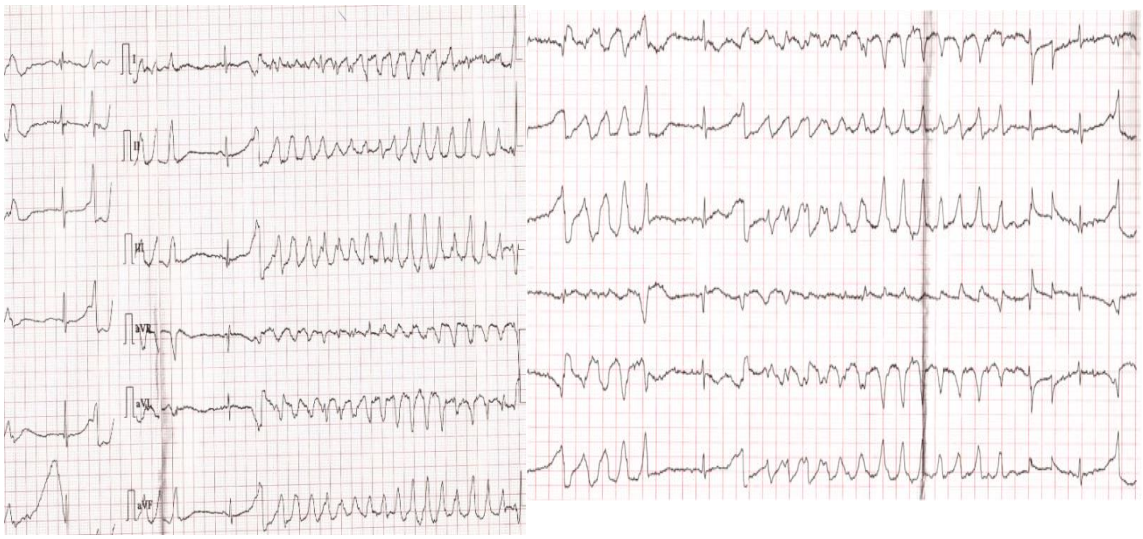
Gastric lavage was applied and activated charcoal was administered through the naso-gastric tube; it were applied volemic, electrolitical and acido-basic repletion and complex clinical – biological monitoring. After 24 hours from admission in ICU II Toxicology, the ECG monitoring registers a new ventriculary fibrillation episode, followed by cardiac arrest. The resuscitation protocol was applied, the maneuvers were efficient. Intensive care measures being were continued, ventilatory support, vasopressor support – with dopamine in the continuous infusion, antiarrhythmic drug – Amiodarone continuous infusion, Lidocaine 1% in infusion. After 96 hours from admission, the patient is hemodynamically stable and the vasopressor support is suppressed; also the patient is weaned from ventilator support. The therapy continued with hepatotrofic and neurotropic drugs, with gastric protectors and general nursing. The evolution was favorable.

Psychiatric evaluation reveals a persistent suicidal idea, depressive facies and a marked depressive disposition, the patient was reffered to a psychiatric unit.

At cardiological reevaluation, 9 days after admission: the patient is hemodinamically stable, arterial pressure 108/77 mmHg, cardiac frequency 77 bpm. Regular rhythm, no murmurs, presence of bilateral pulmonary vesicular murmur, no stasis rales; ECG: sinusal rhythm, cardiac frequency 70 bpm, no ST segment modifications. Cardiac echography: left ventricle not dilated, VSTD 48 mm, not hypertrophied, SIV=PP= 10 mm, no segment kinetics disorders, with conserved ejection fraction 55%, no valve disorders, no pulmonary hypertension; pericardial liquid of 3 mm in diastole, laterally from right ventricle, no hemodynamic impact; diastolic dysfunction – delayed relaxation type.

The patient leaves the hospital toxicologically healed, conscious, cooperative, hemodynamically and respiratoriy balanced; stable from a cardiological point of view under antiarrhytmic treatment with Amiodarone.

Figures 3, 4: Cardiac rhythm abnormalities (episodes of polymorph ventricular tachycardia in the context of long QT syndrome) in the absence of diselectrolithemia, acidosis or hypoxemia after tianeptine overdose.



CONCLUSIONS

Although compared to TCAs, which have been proven scientifically to cause cardiac arrhythmia, the data for tianeptine is limited, the above case reiterates the possibility, even low, but with a deadly risk associated, of malignant arrhythmias being caused by it.

Although proof supporting the hepatotoxic potential of MAOI is limited, overdosing on the aforementioned generated phenomena of hepatic cytolysis, which is indicated by increased enzyme levels.

A correct management of acute poisoning with unclear substances, whether accidentaly or voluntary, involves a precise assessment and promptly initiated therapy.

GC/MS analytical method is the confirmatory method for urinary toxicological screening for a diagnosis of certainty in the case of acute drugs poisoning and is important in order to establish an effective therapeutic course.

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CLINICAL PRACTICE

Article received on October 15, 2018 and accepted for publishing on January 21, 2019.

Principles of antimicrobial therapy in urosepsis: an approach from the perspective of the infectious diseases physician

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Abstract: *Background: This topic is aimed to review the treatment approach in adult patients diagnosed with urosepsis, in order to decrease mortality and to maximize the outcome of the antibiotic stewardship efforts.*

Methods: We present an approach of antibiotic therapy in urosepsis from an infectious diseases physician perspective, based on the recently published data integrating the epidemiology of the urinary tract infections in a multidisciplinary hospital from Romania.

Results: The principles of therapy in urosepsis should have five goals, but not limited to rapid diagnosis, specific intensive care unit treatment, obtaining urine and blood cultures, anti-infective treatment and identifying and correction of underlying risk factors. Due to the higher prognostic value, the antimicrobial therapy in urosepsis should consist in "two steps" regimen: initial antimicrobial therapy, based on "empirical" approach for the first 48 - 72 hours and directed or "definitive" antimicrobial therapy adapted to the susceptibility profile of the isolated pathogens, the penetration into the urinary tract and the patient clinical response. Clinicians should be aware of the different antibiotic susceptibility profile of the bacteria from the hospital compared to the community. Thus, the initial choice of antimicrobial agents depends on several criteria like the severity of illness and the risk factors for multidrug resistant pathogens: the immune status of the patient (age, debilitating chronic illnesses, immunosuppressive treatment or uncontrolled malignancies), broad-spectrum antimicrobial use and health care environment exposures in the previous 3 to 6 months, susceptibility of prior urinary isolates, and local community resistance prevalence of bacteria.

Conclusions: Our important messages highlighted the need of a holistic approach of urosepsis, in a multidisciplinary team, with integration of risk factors for AMR, focusing on the first phase of antibiotic treatment as the main driven of the survival rate.

Keywords: *urosepsis, antimicrobial therapy, infectious diseases, antibiotic resistance*

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INTRODUCTION

In about 30% of the sepsis cases, the underlying condition is related to the urinary tract infections (UTIs). The main risk factors are obstructive uropathies and most frequently, urosepsis develops in the context of health care–associated (HCA) UTIs [1]. The prognosis of urosepsis is more favorable compared to that of sepsis in general, with a mortality rate about 20–40% for severe cases. Unlike etiology in all-cause sepsis, dominated by gram-positive bacteria (GPB), the most common pathogens isolated

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in urinary sepsis are gram-negative bacteria (GNB) [2]. In March 2018, the Centers for Disease Control and Prevention released the "Hospital Toolkit for Adult Sepsis Surveillance", a guidance useful for hospitals to identify sepsis outcomes and evaluate the effectiveness of sepsis prevention efforts [3].

In our perspective, one of the main causes of failure to antibiotic treatment in sepsis is related to the antimicrobial resistance (AMR) and inadequate spectrum of the prescribed antibiotics at the beginning. Therefore, this topic aims to review the treatment approach in adult septic patients with acute complicated UTIs, in order to decrease mortality and to follow the antibiotic stewardship recommendations.

ETIOLOGY AND SUSCEPTIBILITY OF ISOLATED STRAINS

Worldwide, the etiology of urosepsis is dominated by enterobacteriaceae, with Escherichia coli (E. coli) accounting for about 52% of the cases, followed by Klebsiella species (spp), Pseudomonas aeruginosa, Enterobacter spp and GPB, such as enterococci (5%) [4]. In 2017, among the strains isolated from the urology department of our Romanian tertiary hospital, E. coli was encountered in 59.2% of all cases of UTIs, followed by other GNB like Klebsiella pneumoniae (K. pneumoniae) [unpublished data].

It has been reported that the resistance rates of pathogens in urosepsis are higher than 10% for almost all antibiotics. The European Antimicrobial Resistance Surveillance Network for 2016 showed that more than half (58.6%) of the E. coli isolates were resistant to at least one of the antimicrobial groups under regular surveillance (aminopenicillins, fluoroquinolones, third-generation cephalosporins, aminoglycosides and carbapenems) [5].

According to the European Centre for Disease Prevention and Control, the level of E. coli resistance to carbapenems remained rare in 2016 (< 0.1%), with a national percentage ranging from 0% to 1%, meanwhile for K. pneumoniae, data showed large differences, ranging from 0% to 66.9% [6]. Carbapenem-resistant enterobacteriaceae (CRE) represents a significant threat to patients and healthcare systems in all European countries, being associated with high mortality, especially due to the delay in administration of effective treatment and limited treatment options [6]. Despite the fact that in 2016 the E. coli carbapenem resistance level in Romania has dropped from 1.9% to 1%, the reported rates of AMR are very high and rising in comparison to other European countries [7].

MANAGEMENT

In the management of septic patients, we must consider that sepsis is a life threatening condition with a directly dependent prognosis on the timeliness and accuracy of therapeutic intervention. In the latest update of the Surviving Sepsis Campaign (SSC) bundle from 2018, the most important change of the 2016 guidelines was that the 3-h and 6-h bundles have been combined into a single "hour-1 bundle" [8]. Clinicians should initiate antibiotic treatment immediately, especially in patients with hypotension, rather than waiting and extending resuscitation measures. Other measures such as obtaining blood samples, urine and blood cultures, administration of fluids and antibiotics, should all begin immediately [8].

The principles of therapy in urosepsis should follow five rules [9]. First of all, we have to establish a rapid diagnosis based on clinical and paraclinical tests: classical symptoms (dysuria, frequent urges to urinate, flank pain and/or costovertebral angle tenderness, fever, chills, pyuria), quick Sepsis Related Organ Failure Assessment score (qSOFA), biomarkers of sepsis and imaging findings [9].

In addition, a specific intensive care unit (ICU) treatment should be start with fluid replacement, supportive and adjunctive therapy as needed [9]. Obtaining urine and blood cultures prior to antibiotic administration represent another important rule [9].

On the other hand, initiation of antibiotic treatment must be adapted to the patient profile (usually broad spectrum antibiotics) after the quick assessment by the clinician and/or infectious diseases specialist [9]. The last but not least rule is addressed to identification and correction of risk factors (calculus, obstruction, renal or perinephric abscess) by the urologist [9].

ANTIBIOTIC TREATMENT

Broad-spectrum antibiotic therapy aiming to cover all likely pathogens should be started immediately, ideally in the first hour. Data from the literature have shown an association between the rapid administration of antibiotics and improved survival in patients with sepsis. Initiation of empiric antibiotic treatment within the first hour of hypotension is associated with a survival rate of about 80% [10]. Each additional hour of delay was found to decrease the survival rate by 7.6% [10].

The initial choice of antimicrobial agents depends on several criteria like the severity of illness and the risk factors for multidrug resistant pathogens: the immune status of the patient (age, debilitating chronic illnesses, immunosuppressive treatment or uncontrolled malignancies), broad-spectrum antimicrobial use and health care environment exposures in the previous 3 to 6 months, susceptibility of prior urinary isolates, and local community resistance prevalence of bacteria. Clinicians should be aware of the different antibiotic susceptibility profile of the bacteria from the hospital compared to the community. Therefore, an accurate classification of the UTIs according to the place of acquisition of the infection is mandatory. There have been defined two major categories of infections comprising of community-acquired (CA) UTIs or health-care associated (HCA) UTIs with the following scenarios: community onset HCA UTIs or hospital onset HCA UTIs. In CA-UTIs, the possible pathogens are limited usually to susceptible enterobacteriaceae, ampicillin-susceptible Enteroccous faecalis or Staphylococcus saprophyticus. In HCA UTIs, in general we are dealing with resistant strains of bacteria such as extended spectrum beta-lactamases (ESBL)-producing enterobacteriaceae or CRE. It has been shown that inappropriate antibiotic treatment in critically ill patients was associated with decreasing in survival rate of at least 5 times in septic shock [11].

Table 1. Choice of antibiotic therapy in urosepsis

"Empirical" phase of antimicrobial therapy *(combination therapy (ß-lactam antibiotics plus the second antimicrobial agent) is preferred especially in critically ill patients with high risk of death*		
Community-acquired UTIs# *# in some regions the risk of ESBL enterobacteriaceae has to be considered also in community-acquired UTIs*	**Health-care associated UTIs**	
	without risk of non-fermenting GNB or hemodynamically stable patient	*risk of non-fermenting GNB or hemodynamically unstable patient*
Aminopenicillins +/- beta lactamase inhibitor (BLI) (Amoxicilin/ clavulanate) 3rd generation cephalosporins (e.g. Ceftriaxone) Fluoroquinolones (e.g. Ciprofloxacin, Levofloxacin) Aminoglycosides (e.g. gentamicin)	Carbapenems (e.g. Ertapenem) Ureidopenicillin plus BLI (e.g. Piperacillin/tazobactam) Aminoglycosides (e.g. Gentamicin, Amikacin, Netilmicin, Tobramycin) Glycopeptides (e.g. Vancomycin)	Carbapenems (e.g. Imipenem, Meropenem, Doripenem) Carbapenems plus BLI (e.g. Meropenem/vaborbactam) Ceftolozane/Tazobactam Ceftazidime/Avibactam Monobactams (Aztreonam) Colistin Glycopeptides (e.g. Vancomycin) Aminoglycosides (e.g. Gentamicin, Amikacin, Netilmicin, Tobramycin)
"Directed" phase of antimicrobial therapy±,¥		

±according to the antibiotic susceptibility profile of the detected pathogens
¥monotherapy is preferred (exception of critically ill patients with high risk of death and infections with CRE)

The antimicrobial therapy in urosepsis consists in "two steps" regimen:

a) Initial antimicrobial therapy, based on "empirical" approach, should starts ideally, after obtaining blood and urine cultures, during the first hour of the patient presentation in the emergency room or after the diagnosis of urosepis in case of hospitalized patients. Due to the prognostic value of this treatment phase, as a general rule, the broad-spectrum antibiotics with good penetration into the urinary tract are preferred for the first 48-72 h [8];

b) Directed or "definitive" antimicrobial therapy

represent an adaptation of the initial regimen (e.g. de-escalation) according to the antibiotic susceptibility profile of the isolated pathogen, taking into account the clinical evolution of the patient [8]. The duration of definitive therapy should have a minimum duration with maximum efficiency, usually between 10 to 14 days [2];

Based on our clinical experience and the evidence base medicine, we present a personal approach, not validated in clinical trials, regarding the choice of antibiotic therapy in urosepsis (Table 1).

In addition to antimicrobial therapy, an underlying condition such as urinary obstruction, anatomical or functional urinary tract abnormalities (neurogenic bladder, bladder catheters, nephrostomy tubes, and ureteral stents), should be rapidly identified and managed [12]. One of the important issues is related to the appropriate collection of the urine sample in case of urinary tract obstruction. In case of kidney drainage (e.g. percutaneous nephrostomy) we must collect also the urine from the inserted tube in addition to the usual urine sample.

ASSESSMENT OF TREATMENT RESPONSE

Usually, symptoms improve rapidly when antimicrobial treatment is effective, but the fever can persist at least 3 days despite the appropriate antibiotic treatment. The response to treatment is assessed by the following criteria: clinical (fever remission), biological (decreasing levels of inflammatory markers, sepsis biomarkers, alleviating organ dysfunction) and bacteriological (negative urine and blood cultures). In the PRORATA trial, a multicentre randomized controlled trial, it has been evaluated the use of procalcitonin (PCT) to reduce the patient's exposure to antibiotics in ICU. It has been shown that a strategy consisting in measurement of PCT concentration on a daily base allowed for a reduction of antibiotic consumption in ICU [13]. Also, other studies have been proposed a PCT-based algorithm for starting, continuing or stopping antibiotics in patients with sepsis in the ICU [14]. A Swiss randomized controlled trial showed that an algorithm based on PCT concentration and quantitative pyuria measurements, significantly reduces antibiotic exposure, with about 30%, without apparent negative effects on clinical outcomes [15]. In case of persistent or worsening symptoms after 72 hours of appropriate antimicrobial therapy, an additional evaluation is required in order to identify potential factors that might interfere with the favorable clinical outcome. Dosing reevaluation, especially for aminoglycosides and glycopeptides, surgical drainage of the new abscesses, assessment of the inserted tube position and permeability, repeating urine and blood cultures and readjusting treatment to the antibiotic susceptibility profile of other isolated pathogens, should be considered.

CONCLUSIONS

Our important messages highlighted the need of a holistic approach in urosepsis, provided by a multidisciplinary team, with integration of risk factors for AMR, focusing on the first phase of antibiotic treatment as the main driven of the survival rate. The new sepsis "hour-1 bundle" and the principles of antimicrobial therapy should be followed in all emergency departments as a clinical tool intended to improve the general prognosis in urosepsis.

Author Contributions

Concept: VG, AMR; Design: VG, IS, AMR; Supervision: VG, IS, AMR; Materials: CG, FA, AMR; Data collection and/or processing: CG, FA, AMR; Analysis and/or Interpretation: CG, FA, IS; Literature Search: CG, FA, AMR; Writing Manuscript: VG, AMR; Critical Review: VG, IS;

Disclaimer

The authors have no conflict of interest to declare.

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Article received on October 10, 2018 and accepted for publishing on January 20, 2019.

Primary thyroid lymphoma – case report

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Abstract: *Introduction. Even though, primary thyroid lymphoma (PTL) is a rare thyroid malignancy and extranodal non-Hodgkin lymphoma, this type of disease should not be disgraced in order to achieve an early diagnosis and multimodal treatment.*

Materials and method. We selected for discussions a primary thyroid lymphoma patient with absent constitutional (B-type) symptoms, who undergoes a multidisciplinary approach of the disease, chemotherapy and radiotherapy. In this clinical case report a 84 yo male patient with stage IE diffuse large B-cell histological type (Ann Arbor staging) was treated using R-CHOP chemotherapy, followed by involved field radiotherapy, with an excellent treatment response and disease control.

Results and Discussion. Usually this type of extranodal non-Hodgkin lymphoma is present in the seventh decade of life, with males being affected earlier than females. One of the most common presentations of thyroid lymphoma is an enlarging neck mass and a history of Hashimoto's thyroiditis, which is the major risk factor. The most common PTL subtypes are diffuse large B-cell lymphoma (the most aggressive histological subtype, representing 70% of all PTLs) and MALT lymphoma (30% of the PTLs). Advances in both diagnosis and treatment in the last years have improved the early diagnosis and shortened the time for treatment implementation.

Keywords: *primary thyroid lymphoma; non-Hodgkin lymphoma; thyroid cancer; radiotherapy*

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INTRODUCTION

Even though lymphoma is one of the most common hematological malignancies, primary thyroid lymphoma (PTL) is very rare and accounts for 0.5-5% of all thyroid malignancies and about 2% of extranodal lymphomas [1]. Chronic Hashimoto's thyroiditis it is one of the main risk factors known to increase incidence of this type of malignancies [2]. PTL is a rare extranodal lymphoma which is defined as lymphoma that involves just the thyroid or the thyroid and the regional lymph nodes, without metastasis to other areas at the time of diagnosis [3].

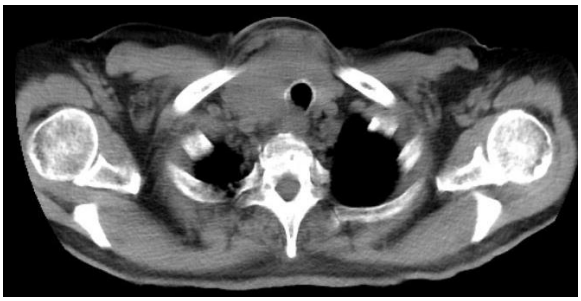
Treatment and prognosis varies according to history and clinical stage. Surgery, chemotherapy, radio-therapy or their combination are the treatment of choice in this particular type of Non-Hodgkin Lymphoma (LNH). Such a case, with PTL, is presented in this article [4].

CASE REPORT

An 84 years old man, known with Hashimoto's autoimmune thyroiditis, with no other relevant personal or family history, visits the oncology department for recent-onset gross neck mass, throat

discomfort, dysphagia and hoarseness (noticed about 1 month before presentation). The patient had no fever, night sweats or weight loss. The physical examination reveals firm, immobile and painless mass descending in the anterior chest wall. Thyroid hormones at presentation revealed high antithyreoglobulin antibodies, an increased thyrotropin level and normal thyroxin. The ultrasound showed a macronodular goiter and chronic lymphocytic cells. On the CT examination of the head and neck region, showed an enlarged thyroid gland, with a nodular structure, starting from the hyoid cartilage and ending in the upper mediastinum (Figure 1). The endotracheal biopsy and immune histological analyses led to the diagnosis of a Non-Hodkin diffuse large B-cell lymphoma (DLBCL), CD20+, Ki-67>90%. A bone biopsy was requested for disease staging, which showed no evidence of neoplazic cells on the examined tissue. Also, the CT scan of thorax, abdomen and pelvis showed no other signs of evolution.

Figure 1 (explication in text)

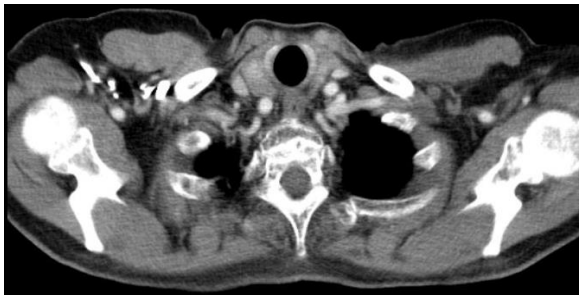


Regarding the fact that only the thyroid was involved (which is an extranodal site, on the same side of the diaphragm) and the fact that the patient had no B symptoms (fever, night sweats or weight loss), the stage of the disease, at the time of the diagnosis, was stage I AE.

After the diagnosis, the patient underwent 4 cycles of chemotherapy with R-CNOP chemotherapeutic protocol, i.e. rituximab (anti CD-20 antibody) 1400 mg, mitoxantrone 14 mg, cyclophosphamide 1350 mg, vincristine 2 mg and dexamethasone 16 mg. The treatment, after 4 cycles of chemotherapy, was stopped because of low tolerance. At the PET-CT scan, following chemotherapy was highlighted high metabolic activity in the right thyroid lobe.

Radiation therapy (RT) was recommended after chemotherapy. A total dose of 36 Gy (18 fractions, 2Gy/fraction) was administrated, using involved field technique to delineate the clinical target volume (CTV). For treatment preparation a fusion between PET-CT and planning CT-scan had to be used for accurate CTV delineation. Three months after radiotherapy, another PET-CT scan was made, to evaluate treatment response, showing low metabolic activity in the right thyroid lobe. The follow-up at 9 months post-chemoradiation was done using clinical examination and a CT scan, which showed a marked regression of the tumor mass (Figure 2).

Figure 2 (explication in text)



DISCUSSIONS

In general population this type of disease is rare, with an annual incidence rate of 2.1 persons per milion [6]. Also, it is known that PTL affects mainly women (5x times more common in women than men) between 50 and 80 years of age, whereas its incidence is low in individuals younger than 40 years of age [7].

We presented a patient with chronic lymphocytic thyroiditis (Hashimoto's thyroiditis), high levels of antithyroglobulin antibodies in different blood tests. These types of patients, with Hashimoto's disease, have a much higher risk of PTL than the general population. It has been presumed that abnormalities in immunity due to an approximately 20-year history of Hashimoto's disease increase the risk of developing PTL [8].

Clinical findings in our patient on presentation, made us consider an anaplastic carcinoma of the thyroid gland, because of the overlapping symptoms. After tumor and bone biopsy, the initial assumption was changed into primary thyroid B-cell lymphoma. The

bone biopsy was made to exclude systemic involvement [9].

The current standard of care is chemotherapy followed by radiotherapy. The role of surgery has been diminished, because an aggressive debulking surgery would not have a more successful response over chemo-radiotherapy [10]. The recommended treatment was 6 cycles of R-CHOP followed by adjuvant RT [5]. In our case it was used R-CNOP chemotherapy because of mitoxantrones lower cardiotoxicity than doxorubicin, but with the same therapeutic outcome as the R-CHOP scheme [11]. RT treatment plan was realized by applying involved field radiotherapy technique, which included in the CTV the whole thyroid gland. A total dose of 36 Gy (2 Gy/fraction, 18 fractions) was administered at the end [7, 12].

An excellent prognosis for early stage PTL (stage IE and stage IIE) is achieved with proper modern treatment management and a multidisciplinary approach. A 5-year survival rate of 87.5% has been observed in patients with DLBCL type. Using modern treatment (combination of chemotherapy plus rituximab agent and radiotherapy), a good response is observed in this type of patients [7].

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CLINICAL PRACTICE

Article received on December 10, 2018 and accepted for publishing on February 20, 2019.

The complex implications of dermatoses specifically associated with pregnancy

Ana Maria A. Stanescu[1], Constantin Stefani[1], Ioana V. Grajdeanu[1], Ioana F. Codreanu[1], Gina Gheorghe[2], Camelia C. Diaconu[1,2]

Abstract: *Pregnancy-specific dermatoses are a heterogeneous group of inflammatory dermatoses associated exclusively with pregnancy. Although these dermatoses are rare, it raises problems in medical practice because of their appearance, being difficult to diagnose, treat and the possibility of the fetus being affected. Pemphigoid Gestationis (PG) is a rare, autoimmune, strongly pruritic, vesiculobullous dermatosis with a strong impact on the patient. Polymorphic Eruption of Pregnancy (PEP) is a benign, self-limited inflammatory skin disorder that usually affects first pregnancy in the third trimester or immediately in the postpartum period. Other dermatoses associated with pregnancy include: Intrahepatic Cholestasis of Pregnancy (ICP), Atopic Eruption of Pregnancy (AEP) and Pruritic Folliculitis of Pregnancy (PFP). We will extensively present PG and PEP, to draw attention to the importance of correct diagnosis and the implications of treatment during pregnancy.*

Keywords: *specific dermatoses in pregnancy, pemphigoid gestationis, polymorphic eruption of pregnancy*

INTRODUCTION

During pregnancy endogenous, immunological, metabolic and vascular complex changes can result in skin damage. The skin changes can be classified into: physiological skin changes, alteration of preexisting cutaneous conditions and specific dermatoses in pregnancy.

Pregnancy-specific dermatoses are a heterogeneous group of inflammatory dermatoses associated exclusively with pregnancy and/ or immediately after birth. Some are considered a significant risk to the fetus.

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These relatively rare conditions, often with variable clinical appearance, and the lack of definitive diagnostic tests have led to confusion in terms of proper diagnosis and management of pregnancy-specific dermatoses.

While many physicians are familiar with normal physiological changes during pregnancy, the knowledge of dermatoses in pregnancy, which is rarely encountered, is often minimal. This review is designed to highlight pregnancy-related

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dermatoses for easier diagnosis and management.

PEMPHIGOID GESTATIONIS (PG)

Pemphigoid gestationis (PG) is a rare, autoimmune, strongly pruritic, vesiculobullous dermatosis, with an incidence of 1: 50,000 to 60,000 pregnancies in USA [1]. The results of European studies suggest a global incidence of 0.5 cases per million people per year [2]. It occurs more frequently in the white race, which may reflect the association with specific HLA haplotypes. Most often, the disease occurs in the second or third trimester of pregnancy, but cases have also been reported in the first trimester and postpartum period [3].

The etiology of the disease is unknown. Nearly half of the cases develop during the first pregnancy. Women with a history of PG are considered to have a higher prevalence of autoimmune diseases, including Graves's disease, Hashimoto's thyroiditis, vitiligo, autoimmune thrombocytopenia, and pernicious anemia [4].

PG is associated with the following antigens: HLA-B8, HLA-DR3 and HLA-DR4 or combinations of HLA-DR3 and HLA-DR4 [5]. The presence of HLA-DR3/DR4 was highlighted in 45% of patients with PG compared with 3% of the general population, and all patients with a history of PG have anti-HLA antibodies [6].

The main antigen of PG is collagen XVII, present both in the skin and placenta, which is exposed to the maternal immune system through an abnormal expression of classical class II histocompatibility molecules in the placenta [7]. PG is exclusively a disease of pregnancy, so potential causes are focused on immunogenicity and potential cross reactivity between maternal-fetal transfer and placental tissue and skin [8]. PG onset in the first or second trimester may lead to negative outcomes, including premature delivery [9].

The diagnosis of PG relies on the clinical evaluation, histological findings, and direct immunofluorescence. Bullous changes are characterized by the formation of subepidermic blister, the bullae contain mostly eosinophils. Eosinophil infiltration is the most common histopathological finding [10].

The inflammatory cellular response of the upper dermis has numerous lymphoid cells, some neutrophils and eosinophilic leukocytes. Microscopic studies have confirmed that IgG is deposited along the basal membrane area, mainly in lucid lamina and located on the proximal anchor filament [11]. IgG class antibodies - the so-called herpes gestation factor (HG) - have an important effect on the genesis of this bullous dermatosis. These antibodies can be transmitted from mother to child, which can cause premature birth, low birth weight, bullous and papular lesions in the newborn; IgG antibodies can be detected by ELISA [12].

Laboratory tests are usually within normal limits. Peripheral eosinophilia may correlate with the severity of the disease, and changes in the level of immunoglobulins, erythrocyte sedimentation rates, acute phase reactants and antithyroid antibodies may occur [3].

A patient who suffered from PG at the previous pregnancy will also suffer from this disease in future pregnancies and the onset of PG will be earlier than in the previous pregnancy. Also, a patient with PG onset in the second trimester may experience remission, followed by exacerbation in the last 6 weeks of pregnancy. The mean duration of the disease at the end of the pregnancy is 4 weeks for bullous rashes and 14 weeks for the urticaria component. Neonatal PG is caused by the transfer of antibodies through the placenta, skin changes in these infants resolve spontaneously within a few weeks and rarely require treatment [13].

The clinical picture is characterized by pruritus, pain, disfigurement, papulovesicular polymorphic eruptions on the abdominal and distal extremities, including the palms, soles, chest and back. The lesions range from erythematous papules, edema papules, confluent vesicles and urticaria plaques through large and tight bullets [14].

Differential diagnosis should be made with: scarring pemphigoid, pruritic urticarial papules and pregnancy plaques, linear IgA dermatitis, multiform erythema, dermatitis herpetiforma, acute urticaria, allergic contact dermatitis, drug-induced bullous disorders,

pruritic pregnancy folliculitis, papular dermatitis of pregnancy, scab [15].

As for treatment, consideration should be given to the fact that PG disappears after birth, but treatment is necessary to minimize pruritus and to prevent vesicles and bullae rash. In mild cases of PG, oral antihistamines and topical corticosteroids will be used, while systemic corticosteroids (prednisone 0.5-1.0 mg/kg/day) will be used in severe cases. Due to prolonged corticosteroid therapy, both the mother and the newborn should be followed to rule out adrenal insufficiency [16]. Alternatives to corticosteroids include azathioprine, dapsone, intravenous immunoglobulin, pyridoxine, cyclosporine, plasmapheresis, minocycline/ nicotinamide, methotrexate and cyclophosphamide. Immunoapheresis is a useful therapeutic option in patients who do not respond to conventional therapy [6, 17].

The clinical course of PG can be altered by changes in estrogen and progesterone levels. Although PG normally recurs in subsequent pregnancies, 8% of cases may not have PG in subsequent pregnancies. 25% of patients may experience relapse when using oral contraceptives or during menstruation. Abortion and premature birth were frequently reported [18].

POLYMORPHIC ERUPTION OF PREGNANCY (PEP)

Polymorphic eruption of pregnancy (PEP), a specific dermatosis of pregnancy also known as pruritic urticarial papules and plaques of pregnancy (PUPPP), is a benign, self-limited inflammatory skin disorder that usually affects first pregnancy in the third trimester or immediately in the postpartum period [19]. The incidence of PPE was estimated to 1 in 160 pregnancies, with the mean age being 29 years [15, 20]. PEP is considered to be the most common dermatosis of the pregnancy [21].

PEP is predominantly present either at the first pregnancy in 70% of cases [15] or at pregnancies that were not performed earlier (for example, spontaneous abortion). The increased incidence of multiple pregnancies in women with PEP has been reported [22]. A specific multiple gestation study in PEP showed that 2.9% of 8000 twin pregnancies had a PEP diagnosis and 14.3% of the triple pregnancies were associated with PEP [23]. It has been shown in several studies that there is a predilection of pregnant women who have PEP to give birth to male children.

Pathogenesis is still unknown, the theory of distension suggests that overstressing of the abdominal wall causes subsequent damage to the connective tissue, triggering an inflammatory response [20, 24].

Infants born from mothers diagnosed with PEP usually do not experience complications or skin manifestations. Premature birth was reported before 37 weeks in 18.4% of PEP patients, compared with 5.8% of patients in the control group [25]. A possible increase in the incidence of cesarean section and premature labor in PEP has been suggested, but there is no clear evidence that it would occur in later pregnancies.

In over 79% of cases, onset occurs in the third trimester of pregnancy, with remission of symptoms after birth, between 1 and 4 weeks [26].

PEP shows papules and pruritic urticarial plaques during pregnancy. Clinical findings were categorized into three PEP subgroups: type I, mainly characterized by papules and urticarial plaques; type II, which included erythematous patches and nonurticarial papules (1-2 mm) or vesicles; and type III, a combination of the other two types. All three types often also have some obstructions with surrounding blanched halos [26]. In terms of distribution, 91% of patients were first found to be the abdominal area, the proximal thighs, and occasionally both [25, 27].

PEP atypical lesions, including vesicles, complicate the diagnosis and have been reported in up to 40% of cases. A retrospective study showed that initially classic clinical manifestations are present in 98% of cases, but 51% of them develop changes in the form of vesicles, target lesions and several eczematous plaques [24]. In less than 2% of PEP patients, it can affect palms and soles, with papulous or vesicular lesions similar to dyshidrotic eczema [25, 28].

PEP is a disorder with a broad clinical spectrum and diverse clinical features, but with absence of pathognomonic identifying features. To exclude other

conditions, laboratory and histopathological investigations will be performed, such as: complete blood cell counts, liver function testing, renal function testing, thyroid stimulating hormone level, urine level, and bile acid level. The differential diagnosis is made with: pemphigoid gestationis, contact dermatitis, toxic drug rash, viral sprain, pitiriasis rosea and urticaria.

Histopathologically, a superficial or deep perivascular dermatitis is found with lymphocytic vasculitis, eosinophils are often present, in some cases, acanthosis, hyperkeratosis and intraepidermal vesicles [29]. If the diagnosis is not conclusive, two biopsies should be performed, one from lesional skin for hematoxylin and eosin staining and one from perilesional skin for DIF. The histopathological examination confirms an inflammatory process underlying PEP and skin stretching has recently been suggested as a precipitation factor for "koebnerization" seen in vitiligo, psoriasis and lichen planus.

It was observed in PEP patients an increase in body weight of more than 15 kg and the infant weight at birth was on average 3.6 kg compared to 3.3 kg in the control group [30]. The prevalence of multiple pregnancies in PEP patients is 10 times higher than in the general population [31]. The skin from PEP lesions showed progesterone receptor immunoreactivity in the cytoplasmic keratinocyte suprabas, while non-lesional skin did not show [32].

Due to the fact that PEP is an autoimmune condition without significant maternal or fetal risk and without recurrence in future pregnancies, patients should not suffer from anxiety due to this condition. Interdisciplinary communication between dermatologist, obstetrician and neonatologist is essential in the management of severe cases of PPE.

Topical corticosteroids are the primary means of treatment for polymorphic eruption of pregnancy (PEP) [33]. The relief of symptoms may require high potency (Class I or II) topical steroids, such as fluocinonide (cream 0.05% / 0.1%, ointment 0.05%) or even systemic steroids (prednisolone 40-60 mg/day). Oral antihistamines can be effective. Symptomatic treatment therapy consists of cooling baths, frequent application of emollients, topical medications containing refrigerated menthol, lightweight cotton, first-generation sedative antihistamines (chlorpheniramine and diphenhydramine) and medically active topical corticosteroids [19, 34].

OTHER SPECIFIC DERMATOSES IN PREGNANCY

Intrahepatic Cholestasis of Pregnancy (ICP) also known as pruritus gravidarum, is a liver disorder characterized by severe pruritus and secondary skin lesions in the third trimester of pregnancy (25–30 weeks gestation). Pruritus typically starts on the palms and soles and later becomes generalized. Secondary lesions such as excoriations, scratch marks, and prurigo nodules might develop as a result of scratching [35]. The symptoms usually disappear 1-2 days after delivery, but is a high risk of recurrence of ICP in subsequent pregnancies (50–70%). ICP is correlated with fetal risks, the most common being premature birth (20–60%), intrapartal fetal distress (20–30%) and stillbirth (1-2%) [20, 36].

Atopic Eruption of Pregnancy (AEP) is a benign pruritic disease with eczematous or papular lesions occurring during pregnancy in patients with a predisposition or history of atopic dermatitis. Specific immunological changes in pregnancy are incriminated in the development of late onset [37]. Clinical manifestations include pruritus, prurigo lesions/ excoriations and eczematous skin lesions, the diagnosis being based on clinical features. Treatment is based on class III or IV topical corticosteroids; in severe cases, systemic or antihistamine corticosteroids may be administered [38, 39]. AEP is not associated with fetal risk factors, except for the child's uncertain risk of developing atopic dermatitis.

Pruritic Folliculitis of Pregnancy (PFP) manifests as small (3–5 mm), multiple, disseminated, erythematous, excoriated, follicular papules and sterile pustules [20]. PFP usually occurs in the 2nd or 3rd trimester of pregnancy (between the 4th and 9th gestation months). Etiology is unclear, has a good maternal prognosis, without recurrence in future pregnancies and without significant fetal harm. Differential diagnosis should be done with acne, bacterial folliculitis, or polymorphic eruption of

pregnancy. Treatment includes topical low-potency corticosteroids, topical benzoyl peroxide 10 %, and ultraviolet B phototherapy [40].

CONCLUSIONS

Most skin manifestations that occur during pregnancy are mild and resolve after birth. A special category is pregnancy-specific dermatoses, which, although rare, can pose great problems in terms of diagnosis, treatment and fetal harm. Good knowledge of these dermatoses is necessary for both gynecologist, dermatologist and family doctor. Proper diagnosis and management are essential for symptom relief and, in the case of fetal risk, adequate monitoring during pregnancy and monitoring of newborns after birth are of major importance. Increased attention should be paid to pregnant patients to understand the risks, adverse effects, and drug interactions of their medications in the treatment of pregnancy-specific dermatoses.

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CLINICAL PRACTICE

Article received on December 10, 2018 and accepted for publishing on January 20, 2019.

Salivary matrix metalloproteinases activity in periodontal disease

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Abstract: *Introduction: Matrix metalloproteinases (MMPs) are key enzymes involved in degradation of extracellular colagen matrix during periodontal disease progression. The aim of our work was to study the levels of MMP-1, MMP-2, MMP-7, MMP-9 and MMP-10 in saliva of patients with chronic periodontitis.*

Materials and methods: The study included 22 patients with chronic periodontal disease and 15 controls. We collect unstimulated whole saliva from all volunteers included in the study. Salivary levels of mentioned MMPs were analyzed using magnetic bead-based multiplex assays and Luminex technology.

Results: MMP-1, MMP-7, MMP-9 and MMP-10 present statistically increased salivary levels at patients with periodontal disease versus the control group ($p<0.05$). Salivary level of MMP-2 at patients with chronic periodontitis was increased but statistically insignificant compared with healthy subjects ($p=0.1$).

Conclusions. The increased level of these enzymes in patients diagnosed with periodontal disease reflects their implication in periodontal tissue degradation. Saliva is an excellent fluid useful in monitoring the severity of this oral disease that affects about half of the adult population around the world.

Keywords: *saliva, inflammation, proteases, periodontal tissue*

INTRODUCTION

Periodontal disease is one of the most common oral disease and affects the adult population around the world. Periodontal disease is a chronic inflammatory disorder that affects the supporting tissues of the tooth, leading to the progressive destruction of conjunctival tissue and alveolar bone.

The role of microorganisms in the etiology of periodontitis has been very well studied, from 600 types of bacteria that are able to colonize oral cavity about 10% plays an important role in the initiation of periodontitis. Porphyromonas gingivalis, Tanerella forsythensis and Troponema denticola, are directly associated with chronic periodontitis. Actinobacillus actinomycetemcomitans, was observed in early stages of periodontal disease and aggressive periodontitis. These

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microorganisms cause periodontal tissue damage either by their degradation products (toxins, enzymes, and lipopolysaccharide) or by activating of the host´s immune system, which leads to the activation of inflammatory cells, cytokine generation, proteolytic enzyme release, and osteoclast activation [1-4].

This inflammatory process is characterized by the increased levels of IL-1α, IL-1β, IL-6, and TNF-α and release of enzymes such as MMPs. MMPs-1, 2, 3, 8 and 9 are key proteases found in biopsy specimens of human inflammatory periodontal tissue [5].

The many advantages of the saliva, namely the rapid, inconvenient collection, anywhere and anytime, the lack of contamination has led many researchers to use it as a new diagnostic fluid for both systemic and oral disorders. Saliva is secreted by major and minor salivary glands, contains 99.5% water, 0.2% inorganic substances and 0.3% organic substances. Salivary proteins include proteins synthesized by salivary glands (mucoproteins and enzymes) and proteins of blood origin, with over 20 protein fractions. Plasma proteins may exceed 20% of salivary proteins and include: albumin, globulin (IgA, IgM, and IgG), α- and β-globulins. The total concentration of γ-globulins is less than 0.5% of the plasma concentration and is generally not affected by the salivary flow. Saliva also contains and non-protein organic compounds such as glucose, lipids, hormones, uric acid, vitamins [6-8].

The main aim of the present research is to detect salivary levels of MMP-1, 2, 7, 9 and 10 in unstimulated whole saliva of patients diagnosed with chronic marginal periodontitis using Luminex technology and Magnetic Bead-Based Multiplex assays.

MATERIAL AND METHODS

Patient selection: We included in our study 22 patients with chronic periodontitis and 15 healthy subjects who agreed to participate voluntary and signed and informed consent. The present research was approved by the Ethics Committee of Science Research from „Carol Davila University of Medicine and Pharmacy". Twenty-two subjects with chronic periodontal disease were recruited from a dental private office in Bucharest. We excluded from our study subjects under 18 years old or with systemic diseases, such as: diabetes mellitus, osteoporosis, chronic hepatitis, liver cirrhosis, HIV/AIDS, autoimmune diseases. All volunteers were diagnosed by the same experienced medical professional.

Clinical data: Demographic data, including age, sex, medical history, medication used and smoking habits (smoker/nonsmoker), were collected. The periodontal parameters performed were: for bleeding index (BI), probing depth (PD), plague index (PI). Chronic periodontitis was diagnosed according to the clinical case definitions proposed by the Center for Disease Control and Prevention.

Samples collection

For all participants in the study, saliva samples were collected after clinical examination. The collection method followed a well-established protocol and took place as follows: 2-4 mL of unstimulated whole saliva was given by each participant in the morning around 9 AM. The participants were asked not eating, drinking or smoking prior to saliva collection. All subjects rinsed with 5 mL of distilled water before collection. Immediately after the collection, the samples were kept on ice and then centrifuged at 5000 rpm for 10 minutes. Samples were aliquoted and stored at -80 ºC until further determinations.

Matrix metalloproteinases detection

All MMPs were detected using Luminex technology and Magnetic Bead-Based Multiplex assays. This method enables simultaneous detection of multiple salivary human biomarkers. Thus salivary levels of MMP-1, -2, -7, -9, -10 were assessed using MILLIPLEX MAP Human MMP Magnetic Bead Panel 2 – Immunology Multiplex Assay. All samples and controls were processed following the manufacturer's specifications. The assay required 25 µL of saliva diluted at 1:20; each MMP had its own detection interval as follows: MMP-1: 27 – 20,000 pg/mL; MMP-2: 68 -50,000pg/mL; MMP-7: 548 – 400,000pg/mL; MMP-9: 14 – 10,000pg/mL; MMP-10: 27 – 20,000pg/mL. The assay involved a 2 hour multistep procedure involving preparation of reagents, samples and controls, preparation of the 96 well-plate and

analysis using a Luminex® 200™ and the xPONENT® software for data acquisition and analysis.

Statistical analysis: The statistical analysis was performed using SPSS software. The characteristics were expressed as median (quartile 1; 3). Statistically significant correlations were found using the Mann-Whitney test (a two-sided p-value less than 0.05 was noted as statistically significant).

Results:

Clinical data: Results for demographic data and clinical examination are present in Table 1. No significant differences were detected for gender and age between the periodontal patients and the control group. Table 2 present the MMPs values obtained in the two groups of patients included in the study.

Table 1. Clinical date of patients included in the study

Variables	Patients	Controls	p-values
Age (*years*)	44.88±12.81	20.67±2	-
Gender (*female/male*)	10/12	10/5	-
Smoking status	0	0	-
BI (*%*)	45±0.11	17±0.20	<0.05
GI (*%*)	59.7±0.24	12.36±0.12	<0.05
PD (*%*)	4.80±0.23	0	<0.05

Table 2. Salivary levels for MMP-1,-2,-7,-9 and -10

Variables	Patients	Controls	p-values
MMP-1 (*pg/mL*)	348.375±144.6	190.125±144.6	<0.05
MMP-2 (*pg/mL*)	116.37±230.12	9.56±230.12	=0.1
MMP-7 (*pg/mL*)	886.68±79.81	527.59±79.81	<0.05
MMP-9 (*pg/mL*)	6885.75±2800.08	5224.911±2800.08	<0.05
MMP-10 (*pg/mL*)	1569.56±168.04	598±168.044	<0.05

DISCUSSIONS

Salivary biomarkers are used in the diagnosis and monitoring of autoimmune disorders (cystic fibrosis, Sjrogen's syndrome), cardiovascular disease, diabetes, HIV, oral cancer, oral lichen planus and periodontal disease [9-13].

MMPs are metal-dependent proteolytic enzymes involved in both physiological (apoptosis, bone remodeling, angiogenesis) and pathological processes such as cardiovascular disease, cancer and periodontal disease [14].

In macrophages and other types of cells from periodontal tissue, lipopolysaccharide from Porphyromonas gingivalis, binds to Toll-like receptor 4 (TLR4) and activates an inflammatory cascade with the release of inflammatory cytokines, chemokines and prostaglandins. This inflammatory response actives inflammatory cells to release MMPs mainly from polymorphonucleate leukocytes (PMN) which will degrade extracellular collagen matrix [5, 15].

Because the extracellular periodontal matrix contains especially type 1 collagen, special attention has been given to MMPs capable of degrading this type of collagen such as MMP-2 MMP-8, MMP-9, MMP-13 [16, 17].

MMPs-1, -2, -8, -9 and -13, had been detected so far in patients with periodontal diseases, mostly from gingival crevicular fluid (GCF) and less from unstimulated whole saliva. The results of the studies reflects increased levels of all mentioned MMPs and their implication in periodontal tissue degradation [18, 19].

MMP-1 or collagenase-1 is the main collagenase found at patients with aggressive periodontitis. GCF and salivary levels of MMP-1 were increased during

periodontal disease and decreased levels were observed after periodontal treatment [20-22].

MMP-1 present increased levels in inflammation, autoimmune and cardiovascular diseases [14, 23].

Our results revealed statistically increased salivary levels of MMP-1 in patients with chronic periodontitis compared with controls.

MMP-2 and MMP-9 are gelatinases that can be released by various type of cells from oral cavity such as gingival fibroblast and gingival keratinocytes [24].

In the present study, we obtained increased salivary levels of MMP-2 but statistically insignificant in patients with chronic periodontal disease versus the healthy group.

The salivary level of MMP-9 was statistically increased in our study at patients diagnosed with periodontal disease versus the healthy group.

Increased levels for MMP-2 and MMP-9 were observed in GCF at patients with periodontitis, versus patients' gingivitis and healthy subjects [25].

MMP-9 in involved in cell apoptosis, tissue remodeling, being associated with several systemic diseases such as systemic sclerosis and osteoarthritis. Meanwhile MMP-2 is involved in angiogenesis and pathological processes such as cardiovascular disease [14, 23].

MMP-7 or matrilysin-1 is produced in non-injured exocrine and mucosal epithelia. Levels of MMP-7 in GCF of patients with periodontal disease are the same with healthy subjects [26].

MMP-7 is associated with inflammation, cardiovascular and lung diseases and to physiological cell apoptosis [14, 23]. Salivary levels of MMP-7 at chronic periodontitis patients involved in our study reflect increased statistically levels compared with controls.

MMP-10 or stromelysin -2 acts on types III, IV and V of collagen and has been linked to cell apoptosis, liver and lung diseases, tissue remodeling [14, 19, 23].

Still now, MMP-10 has not been detected in saliva or GCF in patients with periodontal disease. Our study revealed increased statistically levels at periodontitis patients versus healthy volunteers.

CONCLUSIONS

In conclusion, increased levels of MMP-1, -2, -7, 9, and 10 are directly associated with the severity and progression of periodontal disease, especially through involvement in alveolar bone resorption and periodontal tissue breakdown. Salivary detection of this MMPs is a modern alternative to monitoring the activity of these enzymes.

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Romanian Journal of Military Medicine
New Series, Vol. CXXII, No 1/2019, April
ISSN-L 1222-5126; eISSN 2501-2312; pISSN 1222-5126