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REVISTA DE MEDICINĂ MILITARĂ

- *Evidence based medicine – lessons learned from the NATO Military Medical Center of Excellence*
- *The trend of bladder cancer among Iranian Military Community from 2007 to 2019*
- *Obesity and osteoporotic fractures*
- *Evaluation of the expression level of Sine oculis homeobox homolog 1 in cervical cancer tissue in comparison with healthy adjacent tissue*
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- *Non-melanoma skin cancer (NMSC): Extramammary Paget's disease*
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- *Study on the applications of digital smile design planning technique in complete dentures*
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Contents

REVIEW ARTICLE

Tomáš Vašek, Jaroslav Žďára, Petr Král, Milan Růžička, Michal Potáč, Petr Smola, John Quinn
• ***Evidence based medicine – lessons learned from the NATO Military Medical Center of Excellence*** **153**

ORIGINAL ARTICLES

Yousef Alimohamadi, Mojtaba Sepandi, Alireza Khoshdel
• ***The trend of bladder cancer among Iranian Military Community from 2007 to 2019*** **160**

Florica Sandru, Mihai C. Dumitrascu, Simona E. Albu, Mara Carsote, Ana Valea
• ***Obesity and osteoporotic fractures*** **166**

Ehsan Kakavandi, Ebrahim Faghihloo, Mahdieh Farzanehpour, Mohammad Farahmand, Mehdi Yaseri, Talat Mokhtari Azad
• ***Evaluation of the expression level of Sine oculis homeobox homolog 1 in cervical cancer tissue in comparison with healthy adjacent tissue*** **172**

Viorel Ordeanu, Adrian A. Andries, Lucia E. Ionescu, Marius Necsulescu, Diana M. Popescu
• ***The strategic need for the implementation of a technological platform for the microproduction of antidotes for the CBRN medical protection*** **177**

Lucian G. Eftimie, Daniel O. Costache, Constantin D. Uscatu, Remus R. Glogojeanu, Raluca S. Costache
• ***Non-melanoma skin cancer (NMSC): Extramammary Paget 'sdisease*** **184**

Andrada L. Popescu, Sandica Bucurica, Mihaita Patrasescu, Vasile D. Balaban, Florin A. Savulescu, Mariana Jinga, Florentina Ionita-Radu, Carmen Fierbinteanu-Braticevici
• ***Laparoscopic sleeve gastrectomy and esophageal motility*** **190**

CLINICAL PRACTICE

Mugurel C. Rusu, C. J. Sava, Mihai Stoenescu
• ***Bilateral pneumatization of middle and superior nasal turbinates*** **196**

Ana Maria C. Țâncu, Elena Preoteasa, Alexandra Totan, Ioana A. Guțu, Mihaela Pantea, Marina Imre
• ***Study on the applications of digital smile design planning technique in complete dentures*** **202**

VARIA

Azade A. Farahani, Hamze Shahali
• ***Determine the medical causes and diseases that led to early and permanent medical disqualification of medical cadets*** **208**

Gina Gheorghe, Gabriela Ceobanu, Florentina Gheorghe, Ovidiu G. Bratu, Nicolae Bacalbasa, Simona Bungau, Camelia C. Diaconu
• ***Fever of unknown origin*** **213**

Hamid R. Rasouli, Amir H. Tadayon, Mohammad R. Mashayekhi, Mohsen A. Farajzadeh
• ***Developing a conceptual model for healthcare services branding in the military hospital*** **219**

Diana Ciuc, Dinca Gabi-Valeriu, Peta Dumitru, Marcel Tanțău, Marica Cristian, Achim Florin, Eugenia Panaitescu, Rodica Bîrlă, Silviu Constantinoiu
• ***Redo procedure following Zenker`s diverticulum treatment – a clinical study*** **227**

Guidelines for authors **236**

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REVIEW ARTICLE

Evidence based medicine: lessons learned from the NATO Military Medical Center of Excellence

Tomáš Vašek[1], Jaroslav Žďára[1], Petr Král[2], Milan Růžička[1], Michal Potáč[1], Petr Smola[1], John Quinn[3]

Abstract: *Evidenced-based medicine drives best practices. The Lessons Learned (LL) process in clinical medicine for deployed and military support services drive the NATO Military Medical Center of Excellence (MILMED COE). This review article focuses on the Lessons Learned process and its use in NATO and partner countries medical services. Organizational learning drives development and progress; capturing lessons from mistakes, colleagues' interactions and institutional experience can be lost without an organized lesson learned process. Therefore, in 2014, NATO institutions and Centers of Excellence across disciplines began to focus on the quality management, information sharing and evidence-based practices to maximize outcomes. Since this inception, NATO has implemented the Lessons Learned process and expanded the impact across the alliance in order to save life and prevent illness. The Lessons Learned process and sharing of experience is also a way to improve the quality of care in the military medicine, from preventive, epidemiological, trauma related, casualty evacuation and forward surgical care, among many others, to the medical standardization and organization of military medical services. The Lessons Learned process is an undertaking and an instrument that can also be used to achieve better international and civil-military cooperation. Finally, we deal with the current situation and use of the information obtained from the areas of military health care.*

Keywords: *evidenced-based medicine, knowledge management, lessons learned, Military Medical Service, NATO*

INTRODUCTION – APPLICATION OF THEORY OF LEARNING

Each scientific discipline must be driven by data. One of the areas from which information can be drawn is Lessons Learned, through analysis and a constant cycle of learning and sharing data. This is most applicable in the military medicine and across the healthcare sector, where shared information can be used from various areas, rooted in preventive medicine, epidemiology, traumatology, and others adding to military medicine and healthcare organization and administration.

Institutions that promote health act as microcosms of organism that can evolve through adaptation. As Charles Darwin posited, it's not the strongest survivor, not even the most intelligent, but the one who can best adapt. Every living organism adapts best by learning from past mistakes. In a broader sense, it is about adapting to the environment, as well as the active role of the individual and the choice based on challenges. But learning is not mere adaptation, but it is an active process in which an individual acquires (changes, adapts, and repeats) something that is new. Learning outcomes are actively created personal hypotheses and meanings of reality [1].

There are many theories of learning, but the process we describe in this paper focuses on the theory of Classical Conditioning (I.P. Pavlov), Thorndike's theory of Instrumental Conditioning, Skinner's theory of Programmed Learning, and the theory of Learning through Imitation. Simply put, we

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will do, repeat and develop what we have done and have some positive effect in a particular area. Conversely, we discuss what happened and had some negative consequences that should not be repeated. Based on applying these hypothesis listed above and core knowledge, every organization that wants to survive must behave to the basic strategy of learning from past mistakes.

THE WAY OF THE LESSONS LEARNED PROCESS TO MEDICAL SERVICES OF THE NATO ARMIES

In the early years of the 21st century, NATO understood the importance of transformation and has become a learning organization that has the ability to be constantly flexible and adaptive to remain the central core of European and North Atlantic Security structures in the promotion of safety and security across international communities. For this reason, NATO has issued guidelines outlining the process of learning and sharing experiences, with the clear aim of providing a standardized structure and process that enables this ability in all alliance command structures [2].

Moreover, as outlined within the Armed Forces Declaration, signed by the NATO Heads of State during the NATO Summit, Wales 2014, NATO was directed to enhance the sharing of best practices and lessons learned in support of its Armed Forces personnel and their families [3]. Through the Military Committee (MC), the Committee of the Chiefs of Military Medical Services in NATO (COMEDS) requested that the NATO Centre of Excellence for Military Medicine (MILMED COE) prepare a way ahead to be presented at the 42nd COMEDS Plenary. The proposal was approved and subsequently endorsed by the International Military Staff (IMS) and Military Committee (MC) [4].

LESSONS LEARNED PROCESS AS A PART OF KNOWLEDGE MANAGEMENT

Lessons Learned is defined as a major component of Knowledge Management (KM), not only in the military, but also in government and commercial organizations [5]. The priority objective of Knowledge Management is to achieve higher performance of the organization in fulfilling its tasks in a dynamic, asymmetric and unstable environment (i.e. across the domains of land, sea, air, space and cyber). Therefore, gaining knowledge and experience is a process whereby the Lessons Learned are explored in order to find the best way to deal with the situation and share the outcome to all stakeholders who can benefit from it, and preferably mitigate and reduce mortality and morbidity.

In addition, it is a process allows an organization to learn from its mistakes or use its achievements. Thus, Lessons Learned is an instrument for the continuous process of improving each organization based on evidence [6]. The essence of this process is that it reduces the risk of error repetition in the organization, and the likelihood of repetition of success increases. The introduction of this process within the military health service is important not only for updating the core doctrine that drives best practices, but also for the comprehensive preparation of units in both peacetime and deployed operations.

According to the NATO Bi-Strategic Command Directive 080-006 [7], which is a strategic document of the Lessons Learned process, the following components – the pillars through which the process is implemented – are needed (Figure 1):

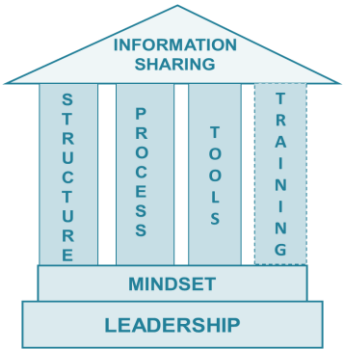
• Processes – links between elements to implement the process, standardize the process to ensure its compatibility and universality both in the NATO structure and in collaboration with other partners.

• Structure – elements (bodies) that are responsible for individual subprocesses and which guarantee an efficient and smooth course.

• Tools – technology, selection and use of appropriate support tools and their optimization to support the Lessons Learned process and its subprocesses.

• Training and education – the main sponsor activities needed to understand the importance of this process.

Figure 1: Components of the Lessons Learned System (NATO, 2016, p. 9)



The ceiling of this process is the very important sharing of information that represents the culmination of the entire Lessons Learned process. However, the most important are the cornerstones of the whole process, without which this process could not work effectively. The base of the Lessons Learned process is made up of commander's support – leadership, with senior military management knowing and understanding the benefits of the process. Then it is the commitment and thought of all involved. It is therefore

necessary to achieve a state where all the participants will understand the importance of this process and, in addition, the support of the commander will be clear to all [8].

The goal of the lessons learned proces

The general objective of the Lessons Learned process is to preserve the organization's knowledge, is to transform the individual's experience into the knowledge of the organization so that other members of the organization can use their experience in the absence of the expert [5].

Therefore, using lessons learned can positively influence other processes of the organization on a continuous cycle of improvement.

Scheme of lessons learned process

The NATO Bi-Strategic Command Directive 080-006 describes the individual parts and sequence of the Lessons Learned process, which is then illustrated in Figure 2.

Observation

This is an identified problem that requires action to be taken to improve the status or best practice for reuse. These are precisely described facts, events, claims, statements that are based on the evaluation of information that describes a certain activity of units, immediate causes, effects or effects.

Knowledge can be Raw Observation, which requires further study or analysis to understand the cause of the event and to supplement the information, or the Mature Observation, to which there is sufficient information, or the cause of the event is known and is not needed its further study and analysis [5, 6, 7].

Analysis

It is a study of the whole through careful examination of its individual parts and their interrelationships [5, 6, 7].

For the purposes of the Lessons Learned process, it is about describing a given phenomenon, its causes and consequences, including the initial design of measures to prevent repetition of inappropriate practices or to make repetition of the appropriate ones.

Lesson identified

Identified experience because of knowledge analysis. In addition to describing the event, the causes and the consequences, it includes a proposal for remedial measures, concrete steps and responsibilities for implementing these measures [5, 6, 7].

Lesson learned

It is the result of the implementation of measures that have increased the ability of a particular element [5, 6, 7].

Knowledge

Newly generated information, whether in the form of knowledge, identified or generalized experience has an individual value for the user corresponding to his/her knowledge (5). Interpreting such information in interaction with experience, facts, individual value, thought processes, and meanings can be understood as knowledge.

Share

One of the most important part of the Lessons Learned process, which is intertwined with the process, is sharing. If NATO really wants to work based on a learning organization, it is necessary for individual Member States to share knowledge with others.

Database of knowledge and experience

In addition, to centrally store, manage, share, and access the knowledge, national and international databases have been created to enable registered users to easily access the information they need.

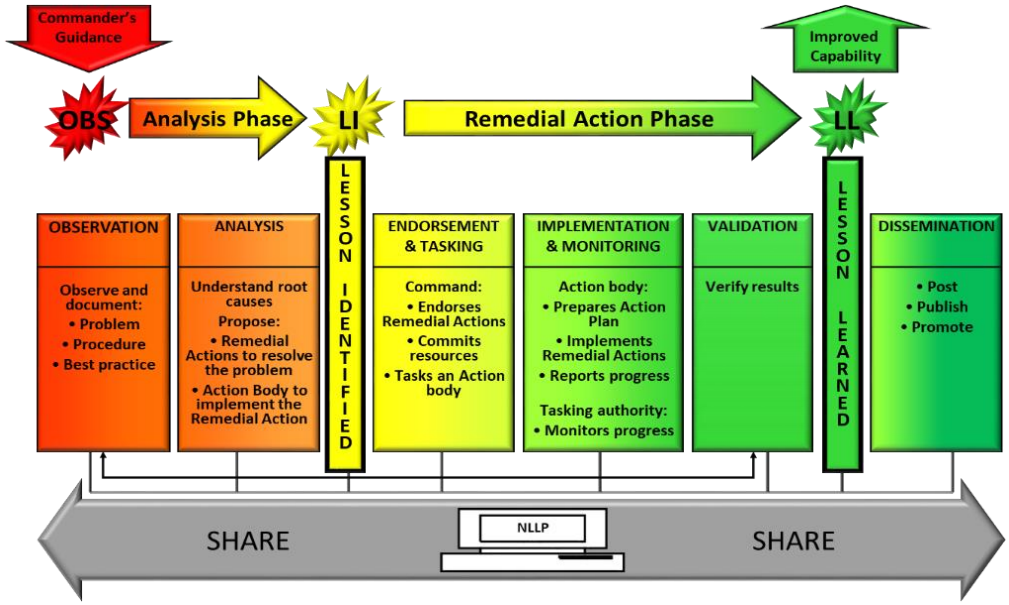
IMPROVING THE QUALITY OF MEDICAL SERVICES ACCORDING TO THE NATO MEDICAL LESSONS LEARNED PROCESS

First, quality in healthcare can be elusive and very complex in the military medical paradigm. The World Health Organization (WHO) defines the quality of health care as the extent to which health services provided to individuals and patient populations improve health outcomes [9]. For this purpose, healthcare must meet these key properties for all. It must be safe, efficient, timely, effective, and fair and focused on people – all activities must be driven by data and evidence based clinical practice. Health care quality can also be explained as a continuous improvement in the quality of health services provided, the ultimate goal of which is to improve health, improve the quality of life and satisfaction of the people they care for [10].

Thus, the Health Care Quality System includes the entire process of creating procedures, collecting information, setting clinical standards and assessing health care, health services and most importantly healthcare outcomes. It is also worth noting that a quality system is an intrinsically interconnected concept of quality elements that should help health organizations meet the goals of their mission [11]. In general terms, this applies to both civilian and military healthcare that the objective of a particular quality system in healthcare is always to improve the quality of health and related care provided in accordance with individual needs

and patient requirements and is rooted in evidence and best practice. In military medical operations, duty of care for warfighters and deployed personnel is the burden of the countries involved and the alliance; this is extended to the combatants and civilian population as outlined in the UN and Geneva Conventions and international norms. This includes the provision of healthcare to all at a standard of care as close to and in keeping with clinical standards provided at peacetime found in the participating countries [12]. This is an extremely difficult task for the provision of medicine for deployed personnel in the dynamic environment that is war, conflict and peacekeeping in remote, austere and resource challenged environment.

Figure 2: Scheme of Lessons Learned
(NATO, 2016, p. 11)



APPLICATION OF LESSONS LEARNED PROCESS IN NATO MILITARY MEDICAL SERVICES

In October 2014, the NATO Center of Excellence for Military Medicine (MILMED COE) hosted the International Security Assistance Force (ISAF), the NATO-led security mission in Afghanistan, Medical Lessons Learned Workshop. This was performed in order to preserve the knowledge gained in ISAF and discuss health care expertise in ISAF in Afghanistan. Examples of the use of appropriate combat application tourniquets during severe limb bleeding have clearly shown that collecting knowledge and sharing Best Practices saves lives and improves the clinical outcomes of those injured on the battlefield. Additionally, on the basis of Lessons Learned from Afghanistan, there was a demand for safe removal by a light armored medical vehicle for evacuation purposes. The MEDEVAC (Medical Evacuation) system was also created based loosely on this process, which can skip individual stages of health care and from point of injury for the warfighter or enemy combatant to ensure they are appropriately evacuated as soon as possible directly to a Medical Treatment Facility (MTF) at a ROLE 2E or ROLE 3 facility.

A number of shortcomings have also been identified in the quality system of medical support; these are of special interest when making the entire system more robust, more resilient and capable of fighting the next battle with potentially different weapon systems and challenges than the current.

The results were presented to the Committee of Military Health Service Chiefs (COMEDS) at their 42nd session. At this meeting, it was decided that NATO MILMED COE would become the main contact and coordination center for the Lessons Learned process within the NATO military health service. Lessons Learned Process for NATO Health Support Centers is illustrated in Figure 3 [13]. In effect, the MATO MILMED COE LL has become the clearinghouse and central crossroads for all thing's best practices in NATO Military Medicine across the alliance.

By positioning itself as the coordinator and collector of observations and lessons, the Lessons Learned Branch of the MILMED COE adds value to the NATO system, processing and sharing Lessons and best practices for all members of the Coalition across the alliance. The LL Branch are building a roadmap to the future of the delivery of Health Support

Services through best practices and rooted in clinical evidence. The members of the alliance have delivered amazing health care, and we need to collect and share the Best Practices across the organization to ensure that everything that has been learned can continue to save lives, financial resources and sparse clinical resources [14].

Currently, the NATO MILMED COE Lessons Learned Branch is developing an inclusive process to improve the collection, processing, analysis, storage and sharing of military health-related knowledge. All the information presented and analyzed is uploaded and stored in the Library of Medical Lessons Learned MILMED COE Information and Knowledge Management (IKM), which is located on the MILMED COE website (http://www.coemed.org/medlldb). An important achievement from the third Medical Lessons Learned Workshop, which took place in 2015, was the establishment of the Multinational Medical Lessons Learned Team (MMLLT), where the Czech Military Medical Service is represented and hosted the second meeting as well. Participating countries agreed to share national information and documents related to military health through the MILMED COE IKM portal. The MILMED COE KM Portal is a good starting point for sharing across the alliance. Each contributing nation should decide on a national level what kind of document they would share. COMEDS has requested to rename the MMLLT, because this name is too similar and thus be confused with Medical Lessons Learned Core Team. With these changes, the new name of the body is Medical Sharing Group.

Figure 3: Lessons Learned Process for NATO Health Support Centres
(MILMED COE, 2015)

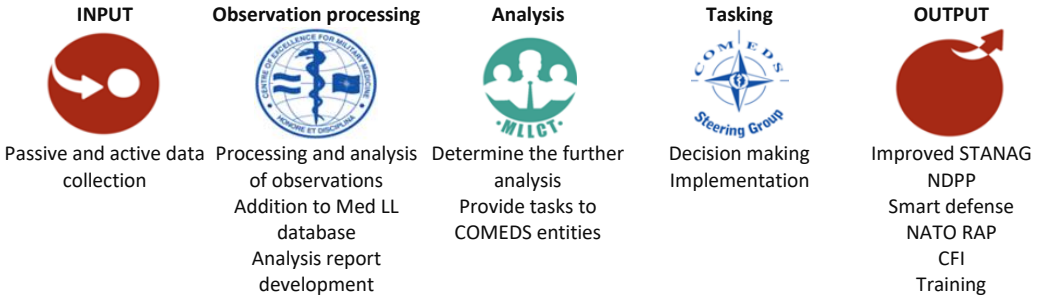
Moreover, the aim of the Medical Sharing Group is to establish the capability to share Lessons Learned information and knowledge across the Alliance [2]. Specifically, the Group members are responsible for reviewing Observations, Lessons Identified, and Lessons Learned information and knowledge within their national or organizational system and for forwarding any item to the NATO Medical Lessons Learned process that is considered beneficial to other Alliance Nations or to the Alliance as a whole. The intent is to empower the exchange of locally identified solutions or Best Practices with the goal of optimizing performance in current and future activities. The type and level of items to be shared will be established by the providing nation or organization and does not need to be the same for every participant. Additionally, the type and level of input provided by a participating entity does not have to remain the same over time and no formal process for change is required, enabling faster sharing of vital information [4]. The Medical Lessons Learned Process, an ongoing effort to improve process, is shown below in Figure 4.

Medical lessons learned conference on civilian – military interaction

A conference on civilian military cooperation has also been held several times. The main aim was to invite civilian and military organizations recently involved in Crisis Management, Humanitarian Assistance and relevant NATO, EU operations and share experiences, best practices and lessons learned. The last event was attended by 36 delegates from 12 nations and 13 organizations. A growing number of Crisis Management and Humanitarian Assistance missions/ events have been resulting in increasing demand for well-organized Civil-Military Interaction. Sharing individual perspectives on the situation and root causes of a crisis is the first step towards de-conflicting actions and complementing

each other's efforts, in effect to remove a duplication of effort. For the successful Civil-Military Interaction sharing of information is key importance to develop a common understanding about the situation and necessary measures.

Figure 4: Medical Lessons Learned Process
(MILMED COE, 2018)



Knowledge and tools of information collection

During the multinational medical exercise Vigorous Warrior 2017 a total of 391 pieces of observations were collected and analyzed. Five focus topics for further analysis were identified: Exercise Development, Interoperability, Patient Evacuation Coordination Cell, Bio-responsiveness and Experimentation [15]. Furthermore, a new tool for collecting and retrieving observations via smartphones was also introduced – the Observation, Discussion, Conclusion, Recommendation (ODCR) application, which was one of the tools for gaining knowledge (also those responsible for collecting knowledge - Office of Primary Responsibility (OPR) LL and paper and electronic forms). This application is fully functional in ANDROID and Apple´s iOS system (free download from Google play and AppStore). The previously mentioned Medical Knowledge Management Portal is a very important tool for preserving and sharing of observations and lessons. Today, there are over 1234 lessons from various areas of military and civilian health care (Exercises, Operations, UN, Ebola and others). Currently, the observations from the latest large-scale international medical exercise, Vigorous Warrior 2019, where 38 NATO and partner countries participated, are being analyzed within these new frameworks. The total number of primary findings is 464, of course, not all of the observations will be uploaded to the KM Portal avoiding repetition, and analysis will follow a standard approach.

DISCUSSION AND CONCLUSION

Evidenced-based medicine drives best practices. Knowledge sharing is an important prerequisite for the functioning and improvement of quality at all levels of healthcare within the deployment of medical units and medical teams across NATO operations. The quality of planning, which often takes place under time constraints and in a hostile environment, is improving and maximizes efforts across the NATO Alliance. The Lessons Learned (LL) process in clinical medicine for deployed and military support services drive the NATO Military Medical Center of Excellence (MILMED COE). This paper describes the Lessons Learned process and its use in NATO and partner countries medical services. Organizational learning drives development and progress and capturing lessons learned improves the quality of care in military medicine on a preventive, epidemiological, trauma, casualty evacuation and forward surgical care basis. The Lessons Learned process is an undertaking and an instrument that can also be used to achieve better international and civil-military cooperation. Lessons Learned process is one of the key elements of developing medical services in line with the changing operating environment.

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Conflict of interests
Authors state no conflict of interests.

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ORIGINAL ARTICLES

The trend of bladder cancer among Iranian military community from 2007 to 2019

Yousef Alimohamadi[1,2], Mojtaba Sepandi[3,4], Alireza Khoshdel[5]

Abstract: *Purpose: Cancers are one of the main causes of mortality in the world. The incidence of cancers in the world and mainly in developing countries is increasing. Due to the importance of this issue, the purpose of the current study was to assess the trend of incidence and death of Bladder Cancer among Iranian Military Community (MC) from 2007 to 2019.*

Methods: This is a cross-sectional study using time series data. All registered bladder cancer in Iranian Military community from March 2007 to February 2017 entered in this study. The future trend predicted using ARIMA (p,d,q) model. All analyzes were performed using ITSM and Excel (2010) software.

Results: According to ARIMA (2, 2, 1) model, the bladder cancer among the Iranian military community had an increasing trend. The AR (12, 2, 0) model showed an increasing trend of this cancer among males but it seems the trend of bladder cancer among females will have a constant trend AR (12, 2, 1). The death due to bladder cancer was showed a decreasing trend using AR (14, 2, 1) model.

Conclusion: In spite of decreasing trend in death due to bladder cancer, the trend of incidence of the Bladder cancer among the Iranian Military Community was increasing. However, it is necessary to adjust the effect of many factors that may have an important role in this trend.

Keywords: *bladder cancer, military community, Iran, Time Series Analysis*

INTRODUCTION

Cancers are one of the main public health problems in the world with different pattern in developed and developing countries. Only 5-10% of cancers are related to human genetics and 90-95% of them are due to exposure to environmental factors and lifestyle [1]. Cancer tumors can affect different organs. One of the common organs involved in tumors is the bladder. Bladder cancer is one of the most prevalent cancers in the world. annually there are many people getting this cancer in the world [2]. Smoking and Infection with Schistosoma haematobium are the main risk factors for the disease [3, 4]. The highest and lowest incidence of the disease was observed in Northern Europe and Asia, respectively [5]. In Iran as a developing country in Persian Gulf Region (southwest Asia), an epidemiologic transition is ongoing from communicable to non-communicable diseases, bladder cancer is sixth most common cancer [6] with estimated age-standardized incidence rate of 10.92 and 2.80 in men and women,

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respectively [7]. In spite of a lower incidence of bladder cancer in the Asian region in comparing to western countries, in Iran, the bladder cancer is one of the most prevalent urological malignancy [8]. according to the results of research, some jobs such as Truck drivers, road construction workers, mechanics, petrochemical workers, plastic and metal industries, welding, and pipeline workers are most prone to getting bladder cancer [9]. To the best of our knowledge there is no study about bladder cancer morbidity and mortality trends among Iranian Military community (active, retired, family, veterans) = MC, so this study performed to time series analysis of bladder cancer in Iranian MC. The trend analysis and prediction of future trends can provide very beneficial information for health policymakers and managers to programing and designing prevention programs. Due to importance of problem the purpose of the current paper was to describe the incidence of registered cases of bladder cancer among Iranian MC during the March 2007 to February 2017 and Forecasting its incidence in the coming years using the Iranian Armed Forces Health Insurance database.

MATERIAL AND METHODS

Study Design

In the current cross-sectional study, the required data were extracted from the registered cases in the Iranian Armed Forces Health Insurance. This data was registered according to the time of diagnosed and registered cases.

Inclusion criteria

All registered bladder cancers in the Iranian MC during the March 2007 to February 2017 entered in this study.

Data analysis

Removing non-stationary in data

Due to the non-stationary nature of the used data set, the needed transformation applied to the used time series. To remove variance fluctuation the Box cox transformation was used. In addition, to remove non-stationary in the mean of data set the differencing was used.

Selection of models

The partial autocorrelation function (PACF) and auto-correlation function (ACF) graphs were used to selection of the prediction model. By using the mentioned graphs the parameters of ARIMA models that include Autoregressive (AR) (p) and Moving Average (MA) (q) were determined.to determine the best model of prediction the different parameters of P and q were tested and the model with minimum Akaike Information Criterion (AIC) statistic was selected to the prediction of the future trend.

Assessing the fitness of the selected models

To assess the fitness of selected model the tests of randomness on residuals that include Ljung - Box statistic, McLeod - Li statistic, Turning points, Diff sign points, Rank test statistic, Jarque-Bera test statistic (for normality) also the schematic checking of the residual graph were used.

Prediction

To prediction of future trends, the ARIMA (p, d, q) model with the minimum amount of AIC was used. By considering monthly time intervals, 120 months from 21 March 2007 to 20 February 2017 were entered into the time series model and the prediction was made from March 20, 2017, to 20 August 2019. All analysis was performed using the ITSM (Interactive Time Series Modeling) software and Excel 2010 by considering the α: 0.05 as a significant level.

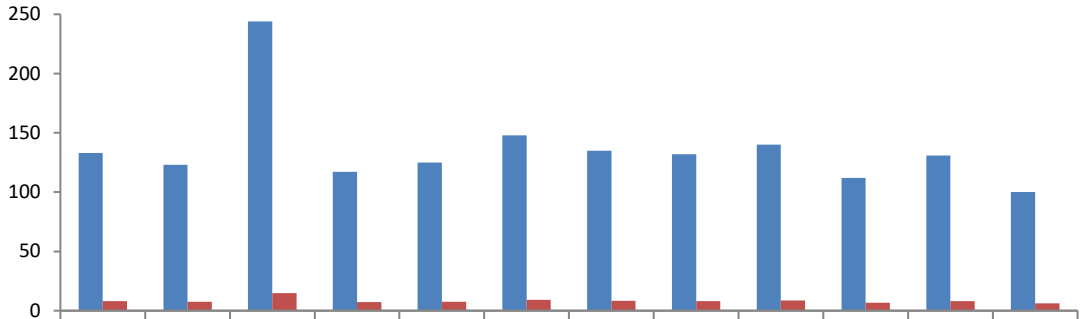
RESULTS

The total registered cases of Bladder cancer during the March 2007 to February 2017 were 1,640 cases. The mean age of registered cases was 70.31±11.63 years. 1,386(84.51%) of total cases were males and 254(15.49%) of total cases were females. Most of cases have had been registered in March and June respectively (Figure 1).

An increasing trend was predicted by a time series Analysis with autoregressive integrated moving average (ARIMA) model with AR = 2, MA= 1, and Akaike information criterion statistics (AIC) = 263 were used (ARIMA [2, 2, 1]) for the coming years (Figure 2).

In addition, an increasing trend was predicted among males (autoregressive model (AR) model with AR = 12, MA= 0, and Akaike information criterion statistics (AIC) = 271 were used (AR [12, 2, 0]). While a constant trend was predicted among females, for the coming years (Figure 4). For the prediction of the trend of this cancer among females the autoregressive model (AR) model with AR = 12, MA= 1, and Akaike information criterion statistics (AIC) = 281 were used (AR [12, 2, 1]) (Table 1). Regarding the prediction of Bladder cancer Mortality among Iranian Military community, the results of time series analysis showed a decreasing trend for the coming years (Figure 5). For the prediction of the Mortality trend the autoregressive model (AR) model with AR = 14, MA= 1, and Akaike information criterion statistics (AIC) = 449 were used (AR [14, 2, 1]) (Table 1).

Figure 1: The registered cases of bladder cancer among Iranian military community in different month.



	January	February	March	April	May	June	July	August	September	October	November	December
n	133	123	244	117	125	148	135	132	140	112	131	100
%	8.11	7.5	14.88	7.13	7.62	9.02	8.23	8.05	8.54	6.83	7.99	6.1

Figure 2: The trend of bladder cancer and predicted value among Iranian military community during 2007-2019.

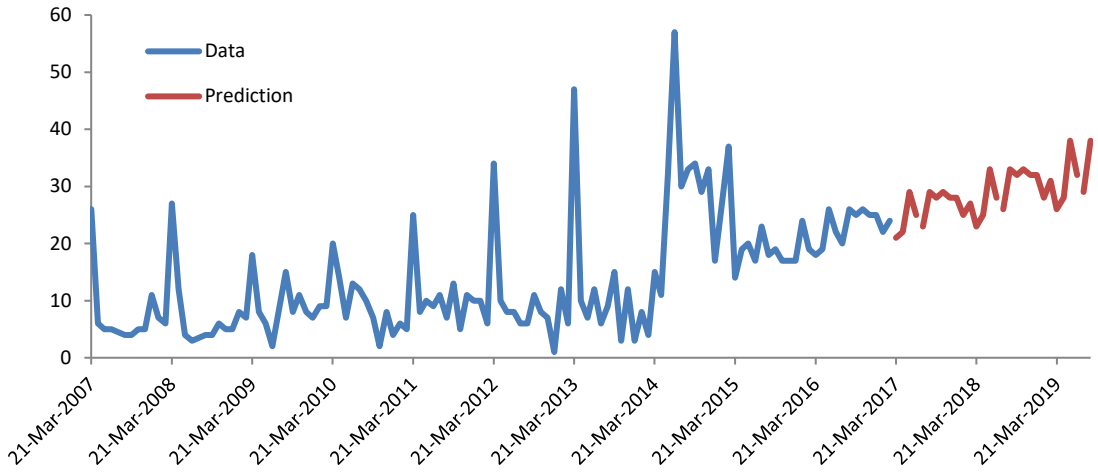


Figure 3: The trend of bladder cancer and predicted value among males of the Iranian military community during 2007-2019.

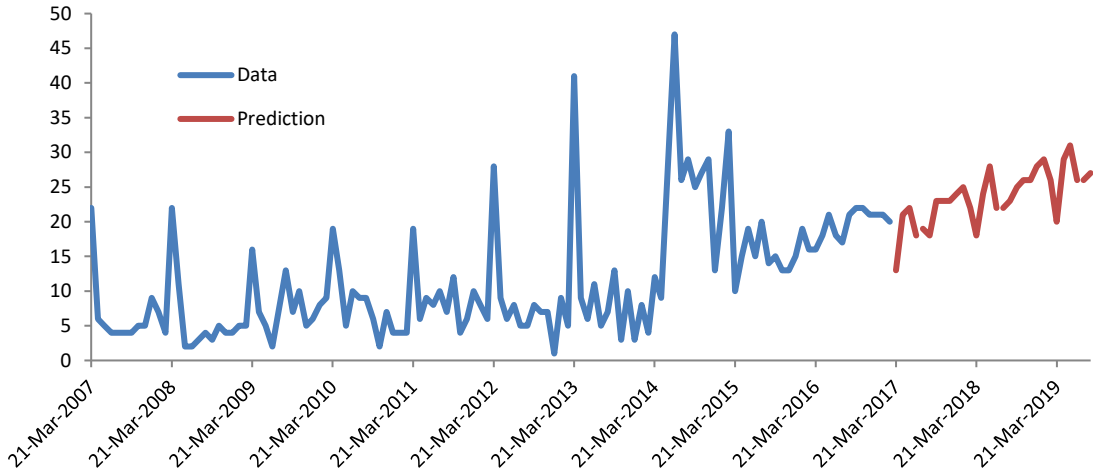


Figure 4: The trend of bladder cancer and predicted value among females of the Iranian military community during 2007-2019.

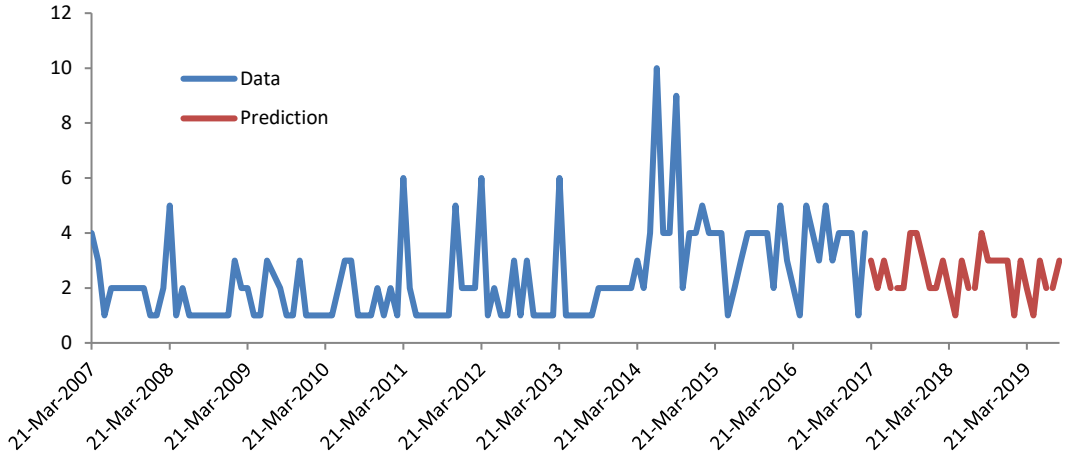
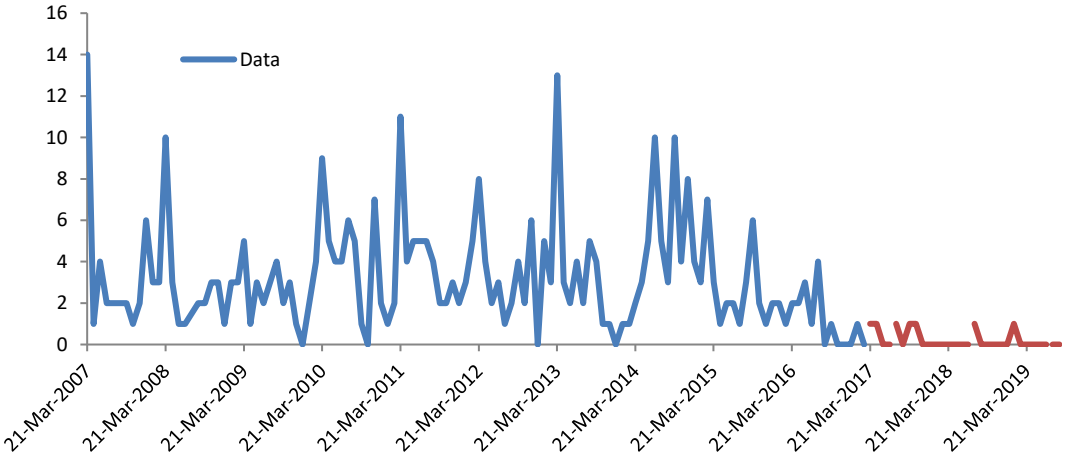


Figure 5: The trend of death due to bladder cancer and predicted value among females of the Iranian military community during 2007-2019.



DISCUSSIONS

Cancer is one of the main causes of mortality in human populations. The high incidence of malignancies may be a consequence of some factors such as aging of the population, sedentary life style, lack of a healthy diet, specific occupational exposures, stress etc [10-12]. Age and gender are related to bladder cancer morbidity [13, 14]. The epidemiological pattern of diseases incidence in developing societies is not similar to that of industrialized nations. Therefore, the investigation and monitoring of overall patterns of malignancies is valuable and it could help public health authorities to trace the etiology and evaluate the effects of diagnostic and screening interventions [15]. Accordingly, the purpose of current study was to assess the trend of bladder cancer incidence and it's mortality among Iranian MC. To the best of our knowledge, no study has assessed both the incidence and mortality rates for Bladder cancer in Iranian MC. According to results of the current study, mean age of registered cases was 70.31±11.63 years. A research in Pakistan reported a mean age of 55.5 years [16] and a study conducted in Iran reported it as to 65.1±12.7 years [5], so it seems the risk of bladder cancer increases with increase in age. The global sex (male/female) ratio for the incidence of bladder cancer is 3.3:1.0. However, it has been reported as 1.1:1.0 in Africa and 5.1:1.0 in Europe [17]. In our sample, this ratio (5.5:1) was comparable to the figures reported in previous research in other studies [5, 16, 18]. More exposure to tobacco, opium, and occupational carcinogens in men may account for the difference in the incidence of bladder cancer between men and women [19]. The time series analysis results showed that the incident cases of bladder cancer among Iranian MC have an increasing trend. Maybe it is due to improving cancer registration system. In addition, changing some lifestyle factors, such as obesity and lack of physical activity should be regarded. Other studies also showed an increasing trend

of bladder cancer in Iran [9, 20] and also in Egypt [21]. Several studies reported an increase in the overall incidence of bladder cancer [22, 23]. However, the trend of incidence may affect by some risk factors for bladder cancer, including tobacco, non-occupational and industrial carcinogens, and population aging [23]. In comparison future trend between males and females, the incidence of bladder cancer among males had increasing trend but the incidence of this cancer among females showed a constant trend. A study by Antoni et al. [24] has studied the global morbidity and mortality trends of bladder cancer. They reported that the incidence rates were diverging, with stabilizing or declining rates for men but increasing rates for females in many countries.

Table1: The related coefficients for prediction incidence of bladder cancer among Iranian military community

	Overall incidence	Incidence in males	Incidence in females	Total death
BOX COX	100	100	100	700
AR	2	12	12	14
MA	1	0	1	1
AIC	263643E+03	271944E+03	281468E+03	449345+03

Some studies showed that obesity and diabetes mellitus is associated with increased risk of bladder cancer [25-27]. However, the results of a recent systematic review and meta-analysis on Iranian military personnel indicate that the prevalence of overweight and obesity in Iranian military personnel is 41% (95% CI: 26.57%) and 13% (95% CI: 10.17%), respectively [28]. So probably, we will oppose to a higher incidence of bladder cancer in the future.

Our study has some limitations: Firstly, all analyses and predictions were performed based on registered data. Therefore, the quality of the registration system could affect the validity of the prediction. For example, to some extent, the increasing trend of incidence may be due to the improvement of the registration system and not related to the increase in the true incidence of cancer cases.

Second, as in most of epidemiological studies we were unable to distinguish between tumor stages because of the nature of the available data. This could reduce the amount of details that can be found from the analyses.

Third, sociodemographic and economic variances can affect the morbidity and mortality of bladder cancer [29]. So lack of investigation about these underlying factors, in the current study could be considered as a major limitation. For example, we don't have any information regarding the size of the covered population by insurance organization of the Iranian military community so the increase in the size of that population may leads to an increase in incidence cases.

Finally, known risk factors, such as smoking habits, obesity, and occupational exposure may affect the interpretation of our results.

In addition, the impact of screening programs on registered cases of cancers should be considered. The improving and developing of such programs may help the diagnosis of cancer cases. Further studies are recommended in the future taking into account the mentioned points, in this regard.

CONCLUSIONS

Our study is the first report on trends of incidence and mortality of bladder Cancer in Iranian MC. Based on the results of this study the need for promoting screening programs is emphasized.

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ORIGINAL ARTICLES

Obesity and osteoporotic fractures

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Abstract: *Obesity correlates with a higher risk of fractures in some sites as vertebras, upper arm, and legs. This is a narrative mini-review focused on several key points that link obesity with osteoporosis. Waist circumference may a better predictor of fractures than body mass index (BMI) in obese females. Even obese menopausal women have an extra source of estrogens in fat tissue this actually is not enough to offer a complete protection against osteoporotic fractures. Low levels of testosterone in males induce a higher risk of fall dependent or independent of sarcopenia (which is more evident in elderly). High BMI is correlated with vitamin D deficiency. Obesity is a contributor to muscle damage and decline due to fat accumulation and inefficient fuel utilisation by the muscle, namely sarcopenic obesity, regardless the age. Associated type 2 diabetes mellitus involves bone damage and fracture risk due to glycated proteins of the matrix, the changes of adipokines, increased cortical porosity, high risk of fall because of blood pressure and glycaemia anomalies, visual disturbances, renal and peripheral nerves diabetes-associated conditions etc. The fracture risk is also augmented via fat derived cytokines and chronic inflammation. Even it seems logical that the weight correction though bariatric surgery reduces the fracture risk in obesity, actually data from longitudinal studies pointed that, despite the procedure is life saving, it does not actually protect against osteoporosis which is regarded as a potential long-term complication. A high fracture burden in obesity should increase the level of awareness for practitioners of different medical areas due to multiple levels of complications and to the epidemiological impact of obesity.*

Keywords: *obesity, osteoporosis, fracture*

INTRODUCTION

Obesity currently represents a pandemic issue and every medical and surgical speciality includes different complications of obesity from cardiovascular conditions, type 2 diabetes mellitus and insulin-resistance, some types of cancer to infections at any level including skin, lungs, urinary tract, gastrointestinal infections, respective infertility, sleep apnoea, non-alcoholic fatty liver disease, depression, and finally any of these or their combination may increase the overall mortality. [1, 2, 3, 4] The increasing epidemiological impact has brought a large area of concern worldwide. [1, 2, 3, 4] The endocrine frame of obesity perception is related to hypothyroidism, hypercortisolemia, male hypogonadism, female hyperandrogenemia, and lately to osteoporosis and fragility fractures, including hypovitaminois D. [5] A systematic review associated with a meta-analysis published in 2019 by van Hulsteijn et al. included 68 studies (meaning 19,996 obese subjects). [6] The study identified that 28% of the individuals had reduced thyroid function of different severities, while 0.9% of them had hypercortisolemia. [6] When it comes to males the prevalence of hypogonadism was between 32 and 42%

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depending on criteria. [6] Contrary to historical records that consider obese women protected against osteoporosis because of the estrogens excess at the level of adipose tissue, last two decades brought enough proves that actually this hypothesis is not entirely true. [7]

AIM

Our purpose is to introduce data related to the risk and prevalence of osteoporotic fractures in obese persons.

MATERIAL AND METHOD

This is a narrative mini-review of the literature based on a research focused on several practical key points that correlate obesity with osteoporosis. A number of 69 articles have been included in the selection.

RESULTS – GENERAL DATA

The influence of abdominal obesity versus body mass index

The link between obesity and fractures may be expressed as BMI (Body Mass Index), either as abdominal obesity. A cross-sectional study published in 2019 showed that among 669 elderly women 65 had fragility fractures of different sites, with an average age of 73.9 years. [8] BMI was not associated with fragility fractures but abdominal obesity suggested by a waist of more than 88 centimetres (cm) was, regardless the presence of different clinical risk factors as smoking or age etc. [8] Some authors suggested that actually the waist circumference is a better predictor of fractures than BMI in overweighed and obese females. [8,9] It seems that low body mass index as well as high content of body fat, respective increase BMI both are associated with high fracture risk, especially at vertebral site. [9] Generally the fat content of the body has been reported as an accurate predictor factor for several conditions including cardiovascular and metabolic morbidities. [10] On the other hand, the influence of BMI on osteoporotic fractures risk may include the fact that low and high BMIs are considered at risk for fractures despite Bone Mineral Density (BMD) values which are directly correlated with BMI and it might not reflect the real fracture risk in obese individuals. [11, 12]

Postmenopausal women

The female individuals in menopause suffer of estrogens deficiency related to menopause thus causing bone loss and low trauma fractures. [13, 14, 15] Even obese menopausal women have a second source of estrogens in fat tissue which is superior to normal weighted subjects this actually is not enough to offer a complete protection against osteoporotic fractures. [13, 14, 15] Some studies pointed a similar rate of fractures in menopausal women regardless they were obese or not and others showed a different distribution depending on site, for instance: a high risk is for vertebral fractures, humerus, upper arm, leg and ankle while a low risk has been described for hip and wrist. [13,14,15] This aspects are most probably related to the physics of fall depending on weight. [13, 14, 15]

Male hypogonadism

Even osteoporosis is less frequent in males, the prevalent fractures are found in obese men through associated hypogonadism independent of the BMI values. [16, 17, 18] Low levels of testosterone induce a higher risk of fall dependent or independent of sarcopenia (which is more evident in elderly). [16, 17, 18] Testosterone therapy may reduce the fracture risk. [16, 17, 18]

Hypercortisolemia

Glucocorticoid osteoporosis is a well known cause of obesity-related type of secondary osteoporosis. [19, 20, 21] The obese subjects without Cushing's syndrome display chronic high values of basal cortisol which seems suppressible at dexamethasone inhibition test. [19,20,21] Chronic autonomous secretion of cortisol is identified in patients with adrenal incidentaloma with an age-dependent incidence and this has been reported to increase the risk of osteoporosis independent of age. [22, 23, 24]

Hypovitaminosis D

The interest in the area of hypovitaminosis D has been explosive during the last decades. A higher BMI is positively correlated with a high risk of vitamin D deficiency based on large epidemiological studies. [25, 26, 27[However, there is still a matter of debate regarding which is the cause and which is the effect. [28, 29, 30] The potential causes of vitamin D lack include: the relative immobility and decreased sun exposure, lower dietary intake, decreased intestinal absorption, high accumulation of vitamin D at the level of adipose tissue since it is a liposoluble vitamin thus a lower bioavailability of the vitamin D. [31, 32, 33] Decreased levels of 25-hydroxivitamin D are contributors to impair muscle resistance and to sarcopenia, thus increasing the risk of fall, and finally the risk of fracture. [34, 35, 36] Also, hypovitaminosis D is a contributor to metabolic disturbances as insulin resistance, hyperlipemia etc. [37, 38] The doses of vitamin D required for supplementation are usually two to three times higher in obese versus non-obese individuals due to fat accumulation. [39, 40]

Sarcopenic obesity

The loss of muscle mass and function is progressive since the

age of 40 and accelerated after 60 years. [41, 42] Obesity is a contributor to muscle damage and decline due to fat accumulation and inefficient fuel utilisation by the muscle, namely sarcopenic obesity, regardless the age. [41, 42] The collateral contributors to this pathological status is represented by low 25-hydroxivitamin D levels and the presence of glucose metabolism anomalies. [43, 44] Overall, the weakness of muscles causes a higher risk of fall thus of fractures. [43, 44]

Type 2 diabetes mellitus

The metabolic anomalies are associated as seen in metabolic syndrome where obese individuals are also diabetics. [45, 46] The risk of fracture caused by type 2 diabetes mellitus is more evident in menopausal women. [45, 46] Type 2 diabetes is associates bone damage and high fragility fracture risk due to glycated proteins of the matrix, the changes of adipokines as leptin, increased cortical porosity, increased risk of fall due to blood pressure anomalies, visual disturbances, renal and peripheral nerves diabetes-associated conditions. [47, 48, 49, 50]

Chronic inflammation

The network between fat and bones includes anatomical aspects like high BMI associates high BMI or mechanical loading is protective for fracture, but also changes of the skeleton micro-architecture in obese subjects that differs with the skeleton site thus some types of osteoporotic fractures are more exposed. [51, 52] Moreover, the fracture risk is augmented via fat derived cytokines and chronic inflammation as seen in metabolic syndrome or abdominal adiposity. [51, 52]

Bariatric surgery

Even it seems logical that the weight correction though bariatric surgery might reduce the fracture risk in obese individuals, actually data from longitudinal studies pointed that, despite the procedure is life saving, it does not actually protect against osteoporosis. [53, 54] Why? Because of the changes involving the mechanical loading due to the weight decrease, of lower intestinal surface for nutrients absorption (including potential malabsorption), anomalies of gut-produced calcitropic hormones, and restrictive diets after surgical procedure etc. [55, 56] Even osteoporosis after bariatric surgery seems a small price to pay, the post-operatory fractures are currently regarded as a long term complication on patients of different ages. [57, 58]

Depression

A higher risk of depression has been reported in obesity. [59] This may further involve reduced physical activity, including decreased sun exposure with risk of hypovitaminosis D and other factors leading to higher risk of fall and fractures. [60] Some mediators anomalies (as well as antidepressants-related) including serotonin have been reported at risk of bone loss through peripheral changes of its concentration despite the fact that circulating 5-hydroxytriptamin has not been yet indicated as a useful bone turnover marker in daily practice and conditions with pathological increase of the molecule as neuroendocrine tumours are not conventionally on the list of secondary osteoporosis. [61, 62]

DISCUSSIONS

Fracture risk assessment

DXA (Dual-Energy X-Ray Absorptiometry) does not particularly reflect the fracture risk in obese individuals since high BMI is correlated with high BMD. [63] FRAX also uses as input BMI. [64] Bone turnover markers might be blunted in associated diabetes and dramatically increased after bariatric surgery. [65, 66] Also type 2 diabetic menopausal women may beneficiate from TBS (Trabecular Bone Score) analysis. [67] The assays of 25-hydroxivitamin D might also be useful. [68]

Current limits

Obese individuals are less likely to receive anti-osteoporotic drugs since they already have several non-osteoporotic complications requiring different therapies. [69, 70, 71] Also, if a specific advisable drug in these subjects should be used in order to treat osteoporosis and/or to prevent fractures is not clearly understood up to this moment which is that drug, and probably the therapy for osteoporosis should be done based on similar protocols as in normal weight subjects with osteoporosis. [69, 71] Moreover, higher doses of vitamin D are necessary in obesity. [69, 71] The importance of therapy in order to provide an adequate primary and secondary intervention regarding osteoporosis is essential since the complications of fractures may become more severe due to obesity co-morbidities. [69, 71, 72]

CONCLUSION

A high fracture burden in obesity should increase the level of awareness for practitioners of different medical areas due to multiple levels of complications and to the epidemiological impact of obesity.

Abbreviations:
BMI = Body Mass Index
BMD = Bone Mineral Density
cm = centimetres
DXA = Dual-Energy X-Ray Absorptiometrry
TBS = Trabecular Bone Scor

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ORIGINAL ARTICLES

Evaluation of the expression level of Sine oculis homeobox homolog 1 in cervical cancer tissue in comparison with healthy adjacent tissue

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Abstract: *Background and Aim: This study aimed to investigate the expression level of Sine oculis homeobox homolog 1(SIX1) in normal and cancerous cervical tissues. Methods: Real-time PCR was used to evaluate SIX1 expression level in fresh surgically obtained cervical biopsies. Also, cervical specimens were tested for HPV DNA detection by MY09/11, GP5+/6+ nested PCR method. Results: The expression level of SIX1 mRNA in Cervical cancer was markedly higher than in normal cervical tissue (p<0.0001). Moreover, HPV DNA was detected in 100% of cases and 21.05% of controls (P<0.0001). Conclusions: This finding indicates that increased SIX1 expression might be of relevance to the pathogenesis of cervical cancer. Perhaps, studies to find agents that specifically target SIX1, develop novel anti-cancer and anti-metastasis drugs. This idea has to be investigated in future clinical studies.*

Keywords: *cervical cancer, SIX1, molecular mechanism, pathogenesis, expression level*

INTRODUCTION

Cervical cancer (CC) is the fourth most common cancer in women with worldwide estimated 527,000 cases and 266,000 deaths in 2012 [1, 2]. Persistent infection with high-risk Human papillomaviruses (HPVs) known as the fundamental cause of CC; although, most of the infected women usually recover spontaneously and only a small proportion progress to CC [2, 3]. HPV E6 and E7 oncoproteins not only inactivate the tumor suppressors p53 and Rb respectively but also modulate a plethora of cellular proteins and signalling pathways related to DNA replication, cell survival, apoptosis, anoikis, epithelial to mesenchymal transition (EMT), anchorage-independent growth (AIG) and angiogenesis. Accordingly, alteration of these cellular cascades leads to cell immortalization, transformation and finally occurrence/development of CC and metastasis [4, 5]. Among these cellular factors, Sine oculis homeobox homolog 1 (SIX1) play a crucial role in CC onset and metastasis [6]. SIX1 is one of the important members of SIX family. This family, including a series of transcription factors, are homolog of the Drosophila sine oculis (so) gene and have a total of six members (SIX1 to SIX6) in vertebrates. Among them, SIX1 has attracted the attention of many researchers recently. SIX1 gene is located on human chromosome 14q23 and its structure composed of two conserved domains, the homeodomain (HD) and the SIX domains (SD). Many studies have shown that SIX1 is essential for the development of

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various human organs in physiological conditions and overexpressed in multiple human cancers, also associated with metastatic phenotype [7, 8]. It is indicated that SIX1 stimulates DNA replication, cell proliferation and maintains survival conditions in CC cells. In the other hand, HPV E7 oncoprotein can induce SIX1 overexpression. Consequently, HPV can obtain opportunity of effectively persistent infection in cervical cells and develop CC [9, 10]. Advanced stages of CC have not effective treatments and most of patients with metastatic CC die. Thus, the better understanding of HPV-induced CC molecular mechanism is indispensable and can assist to introduce more effective novel therapeutic approaches for HPV-induced CC prevention and treatment. In the present study, we evaluate the expression level of SIX1 mRNA by real-time PCR in CC biopsies in comparison with healthy adjacent tissues.

MATERIAL AND METHOD

2.1. Specimens

A total of 38 histopathologically confirmed fresh cervical biopsies consist of 19 cancer samples and 19 normal samples were surgically collected from the Department of gynecology at Imam Khomeini hospital in Tehran, Iran. Normal subjects were women referring to gynecology department of hospital for any cause (for example ovarian cancer), except for cervical involvement. No subjects received preoperative radiotherapy, chemotherapy, or other treatment. The study was approved by the medical ethics committee from Tehran University of Medical Sciences and written informed consent was obtained from all patients before enrollment (approval number 1395.1301).

2.2. DNA Extraction

DNA was extracted from biopsies by the High Pure Viral Nucleic Acid Kit (Roche, Mannheim, Germany) according to manufacturer's instructions. A Nanodrop spectrophotometer (Nanodrop Technologies) was used to determine DNA concentration and purity.

2.3. PCR Analysis

First, we confirmed the integrity of extracted DNA by amplification of a housekeeping gene β-globin (act as an internal control) using PCO3/PCO4 primers. The reaction conditions were as follows: 40 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min and a final extension at 72 °C for 7 min. Then, the presence of HPV DNA was examined by nested PCR assay in all of the positive specimens for the β-globin gene. For this purpose, we used MY09/11 outer followed by GP5+/6+ inner primers (are located within the L1 region of the HPV genome. The Sequences (5 to 3) of all PCR primers are listed in table 1. The temperature cycling program in the first round (MY09/11) and second round (GP5+/6+) PCR was identical as follows: 94°C for 4 min, 40 cycles of 94°C for 1 min, 42°C for 1 min, 72°C for 30 s and a final extension at 72 °C for 4 min [11].

2.4. RNA Extraction

The total RNA from tissue specimens was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The extracted RNA was eluted in 50 µl Diethylpyrocarbonate (DEPC) water. The concentration and purity of RNA were determined by Nanodrop spectrophotometer (Nanodrop Technologies). In order to the prevention of genomic DNA contamination, RNA samples were treated with RNase-free DNase I (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.5. Real-Time PCR

One microgram of RNA samples was reverse transcribed to cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) following the kit's instructions. Real-time PCR reactions were carried out in duplicate with Applied Biosystems 7500 Real-Time PCR system using SYBRs Premix ExTapTM II (Takara, Japan) and specific primers that listed in Table 1 [12]. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was applied as an internal amplification control. The $2^{-\Delta\Delta CT}$ method was used for relative quantification of SIX1 expression levels and results were normalized to GAPDH, as a housekeeping gene.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Variables were compared between groups using the Fisher's exact test or student's t-test as appropriate. Numerical results are expressed as the mean ± SEM. The p-values of <0.05 were considered statistically significant.

RESULTS

3.1. Demographic characteristics

The mean age of the women in the CC group and control group was 61.3 ± 10.2 and 36.0 ± 8.3 years, respectively. Based on statistical analysis, the mean age in the CC subjects was significantly higher than the control group (p-value <0.0001).

Table 1: Sequences of primers used for real-time PCR reactions in current study

Primers	Sequence (5'–3')
β-globin	PCO3 (Forward) ACACAACTGTGTTCACTAGC PCO4 (Reverse) CAACTTCATCCACGTTCACC
HPV	MY09 (Forward) CGTCC(A/C)A(A/G)(A/G)GGA(A/T)ACTGATC MY11 (Reverse) GC(A/C)CAGGG(A/T)CTATAA(C/T)AATGG GP5+ (Forward) TTTGTTACTGTGGTAGATACTAC GP6+ (Reverse) AAAAATAAACTGTAAATCATATTC
GAPDH	(Forward) GGAGCGAGATCCCTCCAAAAT (Reverse) GGCTGTTGTCATACTTCTCATGG
*SIX*1	(Forward) AAGGAGAAGTCGAGGGGTGT (Reverse) TGCTTGTTGGAGGAGGAGTT

HPV, Human papilloma virus; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; SIX1, Sine oculis homeobox homolog 1

3.2. Prevalence and type of HPV infection

HPV DNA was detected in 100% (19/19) of the cancer cases and 21.1% (4/19) of normal cervical specimens (Table 2). There was a significant difference between the two studied groups (p<0.0001). After sequencing analysis of HPV-positive specimens, we identified HPV-16 in all of the CC samples. Also genotyping of 4 HPV-positive specimens in normal group was HPV-16: 2 cases, HPV-66 and HPV-68: each one was one case (Table 2).

Table 2: Demographic and clinico-pathological characteristics in patients with cervical cancer and without cervical cancer

Variables	Cases (n = 19)	Controls (n = 19)	p-value
Age (mean years)	61.3 ± 10.2	36.0 ± 8.3	<0.0001, t-test
HPV positivity	19 (100%)	4 (21.1%)	<0.0001, Fisher'sisher's exact test
HPV type	HPV-16 = 19	HPV-16 = 2, HPV-66 = 1, HPV-68 = 1	

HPV, Human papilloma virus

3.3. SIX1 mRNA expression

Relative real-time PCR results showed that the mean SIX1 mRNA expression in the CC patients was 2.61 ± 0.09 and in the normal subjects was 1.12 ± 0.03 (Table 3).

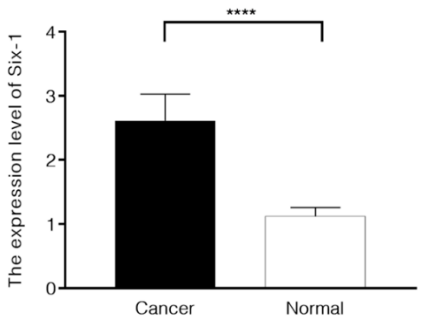
Table 3: Expression level of SIX1 in patients with cervical cancer and without cervical cancer

Gene		Cases	Controls
*SIX*1	Mean ± SE	2.61 ± 0.09	1.12 ± 0.03
	CI	2.42 - 2.79	1.06 - 1.18
	Minimum expression	1.94	0.92
	Maximum expression	3.47	1.39
	p-value	<0.0001 t-test	

SIX1, Sine oculis homeobox homolog 1; SE, standard error; CI, confidence interval

Then, statistical analysis revealed that SIX1 mRNA levels were significantly (p<0.0001) higher in CC tissue compared to the normal cervical tissue (Figure 1).

Figure 1: The expression level of SIX1 in CC and normal cervical group. Data are presented as mean ± SEM and the fold increase of transcripts for SIX1 mRNA normalized by GAPDH housekeeping gene. ****p<0.0001.



DISCUSSION

The sine oculis homeodomain (SIX) proteins are a family of evolutionarily conserved transcription factors that play a pivotal role in both embryogenesis and carcinogenesis. The

homeoprotein transcription factor SIX1 is one of the members of this family that has attracted much attention from the scientific community in recent years [13]. Based on the previous studies, SIX1 upregulated in several human cancers such as breast, ovarian, cervical, liver, rhabdomyosarcoma and Wilms' tumor, also contribute to initiation and metastatic development of these malignancies. Researches have also observed the SIX1 overexpression strongly correlated with poor prognosis and shortened overall survival in cancer patients [14-16]. Consistent with previous studies, we demonstrated the SIX1 mRNA levels is significantly higher in CC than normal cervical tissues. The results of previous studies showed that SIX1 can drive cancer stem cell phenotype and EMT, also lymphangiogenesis, consequently metastasis via stimulation of the TGF-β signaling pathway and VEGF-C, respectively [17-19]. EYA2 act as a coactivator for SIX1, thus mediate its transcriptional activation. Previously it has been shown that inhibition of SIX1-EYA2 complex formation, may be a promising and more effective approach for treatment of cancer and tumor metastasis [20]. In CC cells, SIX1 act as principal regulator of DNA replication, as well as a key factor in metastasis process. Therefore, SIX1 upregulation can lead to malignant proliferation of cervical cells, also metastasis through stimulation of DNA replication-related genes and a5b1 integrin, respectively [10, 21]. Interestingly, high-risk HPV employ various mechanisms to promote metastasis in CC [4]. Anoikis, meaning homelessness, is a detachment-induced cell death. Anoikis resistance, EMT, AIG, and angiogenesis are important cellular events that closely associate with cancer metastasis [22]. Hr-HPV E7 oncoprotein can stimulate SIX1 overexpression in CC. SIX1 consider as an EMT-activating factor, thus its upregulation result in EMT through increasing of ZEB1 and decreasing of E-cadherin. Indeed, SIX1-induced EMT, increase cell survival in detached cancerous cells and drive anoikis overcoming and finally, metastatic dissemination [9]. In the present study, among the total of 38 fresh surgically obtained cervical biopsies, 100% (19/19) and 21.1% (4/19) were positive for HPV DNA, in CC and normal adjacent groups, respectively. Then, sequencing analysis revealed all of HPV-positive specimens in CC group were HPV type 16 that highlighted the indispensable role of HPV-16 in CC onset and progression. The previous studies demonstrated the high risk HPV-16 and HPV-18 account for about 70% of CCs [23]. In current study, four HPV-positive specimens in normal cervical group were: two samples of HPV-16 and one sample of HPV-66 and HPV-68. Based on international agency for research on cancer (IARC) classification, HPV-66 belongs to 2B category (probably carcinogenic). Also, HPV-68 belongs to 2A category (possibly carcinogenic) [24]. Our outcomes corroborated previous reports specifying that the SIX1 involved in the tumorigenesis of cervical cancer [13, 25]. The main limitation of our work was low sample size. In conclusion, SIX1 upregulated significantly in CC, which in turn induces TGF-β, VEGF-C and ZEB1 that collectively can have an association in the tumorigenesis and metastasis of CC.

CONCLUSIONS

We demonstrated SIX1 expression levels was frequently expressed in CC tissues. Also, HPV DNA was detected in 100% (19/19) of the cancer cases and 21.1% (4/19) of normal cervical specimens. HPV can upregulate SIX1 in CC. In addition, SIX1 can contribute to the development of CC and lymph node metastasis of cervical tumors. Nevertheless, the precise biologic role of SIX1 in CC is unclear yet and future works should study on the larger number of patients.

List of abreviation:
CC, Cervical cancer; HPV, Human papillomavirus; SIX1, Sine oculis homeobox homolog 1; EMT, Epithelial to mesenchymal transition; AIG, Anchorage independent growth; so, Drosophila sine oculis; HD, Homeodomain; SD, SIX domains; DEPC, Diethylpyrocarbonate; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; IARC, International agency for research on cancer.

Contribution Note:
EK, EF, MFP, TMA designed the study, EK, EF, MFP performed laboratory works, MFP, MF, MY statically analyzed the data, EK, EF, MFP, MF, MY, TMA wrote and revised the manuscript.

All authors read and approved the final manuscript.

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ORIGINAL ARTICLES

The strategic need for the implementation of a technological platform for the microproduction of antidotes for the CBRN medical protection

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Abstract: *The "volatile" international context requires the strengthening of the national security measures, including in the field of CBRN and public health. The medical countermeasures for the defense are based ultimately on the prophylactic treatment (pre-exposure, intra-exposure and post-exposure) and on the curative treatment (of emergency, maintenance and recovery) of the contaminated and the sick. Depending on the rapid and correct etiological diagnosis, the appropriate treatment is immediately applied. Although in times of peace this scenario is almost non-existent, during war or terrorist attack it can be a mass pathology, like an explosive epidemic, that requires specific therapeutic means.*

Military medicine had and has the conceptual capacity to make antidotes for CBRN medical protection; some have been patented and/or produced as experimental models, but it has no manufacturing capacity anymore.

Keywords: *medical protection, antidote, microproduction, technological platform, medical countermeasures, pharmaceutical technique, orphan drug production*

INTRODUCTION

The international context following the conclusion of the "Cold War" paradoxically requires the strengthening of national security measures, including in the field of CBRN and public health. The international context shows that although the risk of biological warfare has been reduced following the Geneva Convention (Biological and Toxins Wapons Convention – BTWC 1972), signed and ratified by about 90% of the world's countries, the risk of bioterrorism is real, as is the risk of pandemics, zooanthroponosis etc.

The World Health Organization (WHO), the European Union (EU) and the North Atlantic Treaty Organization (NATO) recommend concrete measures to reduce these risks and to increase the responsiveness of each country, of the alliance and of the international community. Therefore, in this context, it is absolutely necessary to implement cutting edge technologies for diagnostic and prophylactic treatment, taking into account the mechanism of action in infectious diseases, the therapeutic means of counteracting the effects of biological agents and the epidemiological surveillance.

The existing international concerns force us to align ourselves with the situation existing worldwide. Designing and equipping such platforms according to international recommendations, determines the level of performance of such infrastructure to contribute to the worldwide effort of medical protection against biological agents. The present study was performed at the request of the Medical Directorate, following the request of the Control Body of the National Ministry of Defence.

MEDICAL COUNTERMEASURES

The medical countermeasures for CBRN defense are based ultimately on the prophylactic treatment (pre-exposure,

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intra-exposure and post-exposure to the attack with pathogens) and on the curative treatment (emergency, maintenance and recovery) of the contaminated and the sick. Depending on the rapid and correct etiological diagnosis, the appropriate treatment is applied immediately. In times of peace this case is almost non-existent; however, during war or terrorist attack it can be a mass pathology, like an explosive epidemic. that requires specific therapeutic means. Medical intervention requires the same strengths and the medical means all at the same time, so medical aid becomes inefficient in quantity. Moreover, the necessary drugs, in principle the specific antidotes, are orphan drugs, being little used because they are addressed to very rare diseases, and therefore they are manufactured in very small quantities and are not available when needed for urgent treatment for a large number of patients.

The medical protection against the effects of the weapons of mass destruction: chemical, biological, radiological and nuclear (CBRN WMD) is based on fundamental and applied scientific research (CDI) and later on its application in practice.

Critical situations caused by CBRN weapons/agencies are major medical emergencies and can be caused by CBRN WMD attacks, terrorism or criminal acts. The medical system must immediately have at its disposal the necessary force and means of special facilities and appropriate procedures, because "later it may be too late".

Antidotes are specific drugs for the emergency, prophylactic and curative treatment of diseases caused by physical, chemical or biological harmful factors, including CBRN agents, pollutants, etc. There is a risk of using a large variety of WMD agents: chemical warfare agents (CWAs), biological warfare agents (BWAs), radiological warfare agents (RWA), industrial toxicants, etc. which cause a wide range of diseases. As a result, many types of antidotes are needed, permanently available in stock. These must continuously exist, in appropriate forms and in reasonable quantities, for the emergency treatment of the contaminated, the suspected and the sick among the military and civilians, and possibly for the treatment of service animals, etc. In this category can be included decontaminants for internal and external use for humans and animals, in the form of solutions, powders, etc.

In medical practice, antidotes, in a broad sense, can be classified according to several criteria. WHO 20 list of essential medicines, 2017 edition, includes at chap. 4. Antidotes and orher substances used in poisonings, only 15 pharmaceuticals with antitoxic effect, but without taking into account the other CBRN agents. Antidotes can be classified pharmacologically, depending on the agent for which they are used, hence etiological. Antidotes can also be classified according to clinical use: preventive (eg protectors, vaccines), post-exposure prophylaxis (eg atropine, specific antibiotherapy, decontaminants for the skin, mucous membranes, eyes etc.), or therapeutic (e.g. decorporators, chelators, therapeutic sera). From a pharmaceutical point of view, there are antidotes for external use, including hygiene-cosmetics (solutions, lotions, pastes, ointments, powders, etc.), antidotes for internal use (buccal solutions, powders, granules, tablets, dredges, capsules etc.) used preventively, post-exposure or therapeutically, as the case may be. Injectable antidotes (specific vials and devices, self-injecting syringe, auto-injector) and infusions represent an important category in the medical-military arsenal of countermeasures against CBRN agents.

Like any other drug, antidotes can be prepared industrially, galenically or masterfully, from natural and/or artificial pharmaceuticals. These can be of mineral origin (eg sodium sulfate), vegetable (eg charcoal, atropine), animal (anti-venom), microorganisms (eg vaccines, immuno-modulators). Depending on the context, they can be used for prophylaxis (eg pyridostigmine bromide, vaccines), for post-exposure prophylaxis (eg decontaminants for skin and mucous membranes, atropine, anatoxins, antibiotics), curative (eg EDTA, therapeutic sera, antimicrobial chemotherapics).

Antidotes must meet a number of basic conditions required by the Medicines Law: proven therapeutic effect, undesirable side effects as less as possible, preparation in appropriate conditions, reasonable cost price, the possibility of being produced in the required quantities and to be stored for a certain time period, as well as being distributed in a timely manner for emergency treatment. However, an issue arises concerning the cost/efficiency: if within the antidote's validity period there are no cases or there are very few (desirable situation), the remaining antidote must be discarded at the expiration of the term of validity. These stocks cannot be refreshed by "being put in use" in the sanitary units, because under normal conditions they are of no use except to a very small extent. As European Medicines Law no longer allows re-endorsement after expiry, at most, the antidotes that correspond to the pharmaceutical and pharmacological control may be used only for possible veterinary use, if applicable. In the event the numbers of cases require a certain antidote exceeding the existing stock, the product will have to be prepared quickly, according to a product portfolio located at the manufacturer. If there is no authorized manufacturer, a call can be made for emergency imports or international aid. In fact, the EU Charter also

provides for a solidarity clause, whereby European countries are obliged to help each other in case of emergency, but it is obvious that this can be done only after covering their own needs.

In practice, it is difficult to find a pharmaceutical industry producer (one of the most profitable in the world) to manufacture the full range of antidotes, because the orders are small, the products are "niche", and the prices are monitored. This is because antidotes are orphan drugs, being used only for rare diseases, so the demand is so low that their manufacture would mean a lot of work for little money, and would not be cost effective. It turns out that a private producer could not be forced to manufacture them in normal circumstances.

The production of specific antidotes for CBRN medical protection can be achieved only in conjunction with:

1) the medical research's need to physically manufacture the newly designed products (medicines, diagnostic kits, kits and medical devices), at the level of experimental model (EM), functional experimental model (FEM), prototype and/or technological demonstrator, for endorsement, receipt and possibly patenting (as an invention or industrial model), with

2) the need of the industry to carry out the 0 series, the microproduction and the batch series production for clinical testing and for obtaining the marketing authorization (MA), so the principle of integration must apply: research, education and production.

This integrative principle promoting success in the development of specific antidotes, was introduced in Romanian medicine by Prof. Ioan Cantacuzino, since 1901, (Biography Cantacuzino, Ciuca) being applied at the Institute that bears his name, but was subsequently abandoned under unjustified circumstances. Obviously, nowadays, such a dedicated objective involves a pharmacological and pharmaceutical collaboration in the form of a technological platform for the production of antidotes for medical protection, whose components (facilities and utilities) include a Pharmaceutical Laboratory for CBRN antidotes and that must comply with the recommendations of the ANMDM (National Agency for Medicines and Medical Devices) and implicitly with those of the WHO.

State-of-the-art for the production of antidotes for CBRN medical protection is different from one country to another. Civil drug manufacturers (the pharmaceutical industry) generally avoid manufacturing antidotes, as these are "orphan drugs", meaning that they are rarely used under normal conditions, so it would be a difficult commodity to sell. State producers can be forced, through the Production Plan, to manufacture them, but in Romania there is no longer the case, as a result of privatization, and private producers risk financial losses or even bankruptcy, if they manufacture without and end profit.

The beneficiaries and the producers avoid ordering or storing antidotes, because they can remain unused until the expiration date and the prolongation of the duration is not allowed even if they maintain their therapeutic effect, so the medicines are canceled and financial problems arise. As a result, CBRN-specific antidotes do not appear as such in the List of Essential Medicines (WHO Model List of Essential Medicines, edition 21, WHO 2019).

The solution would consist in the specific pharmaceutical manufacture in military or militarized facilities, according to the needs of the national military forces, of the population exposed to specific risk and possibly for other allied armies or for the population in general.

PREVIOUS EXPERIENCE

In Romania there was the Pharmaceutical Production Laboratory of the Medical Directorate belonging to the Ministry of National Defence, which operated without interruption for almost a century, near the Sanitary Warehouse. It prepared specific usual medicines for the military staff, which were distributed free of charge to military hospitals and unit nurses. Different products were manufactured, depending on the needs, as solid forms (tablets, dragees, suppositories, spark plugs, powders), liquid forms (solutions, lotions), ointments, etc. But the injectable, infusable forms and the eye drops, which must be sterile, were prepared only in the pharmaceutical laboratories of the military hospitals. These drugs, along with others, manufactured in the country or imported, have been used to treat the military during peace and war: the Balkan Wars, the First World War, the Second World War, the Revolution, etc. After Romania's entry into NATO and the EU, this laboratory was closed by the pharmaceutical micro-production and moved to another headquarters because it did not comply with the new European norms for the pharmaceutical industry. At the entry into force of the Medicines Law, it was closed down and the machines, inventory objects and consumables (pharmaceuticals and chemicals) were redistributed, some of them ending up at CMMSR for scientific research. They no longer meet the conditions required for the pharmaceutical industry, can no longer be used for the initial purpose and cannot be exploited.

The laboratories of The Center for Military-Medical Scientific Research (CMMSR) have experimentally produced various

specific drugs, including antidotes, some of which have been used for the troops in peace or war time. These include: Dostirav (Pyridostigmine) and other CBRN protective equipment provided, including in the Iraq war, 1991; the cyanide antidote that, although patented, is not manufactured by the pharmaceutical industry and the tragic consequences could be seen in the tragic accident which took place at “Colectiv” etc.

The applied research, through the module "Scientific and technological research on the production of antidotes and products with special purpose", has completed technological researches on the manufacture of protective and decomposing antidotes under conditions of peace and in critical conditions (campaign, natural and technological disasters) and, since 2002, ended by:

- establishing the technologies for industrial production and by micro-production under critical conditions for oral preventive antidotes (eg Dostirav/30 mg);

- establishing technologies for industrial production and by micro-production under critical conditions for decontaminants for skin and the mucous membranes (eg DCPM);

- establishing the structure and operating conditions of certain microproduction lines under critical conditions for antidotes;

- research on the synthesis of some organic derivatives of obidoxime with improved pharmacokinetic properties, etc.

These research projects were approved and accepted, the microproduction was performed at galenic level, and the financing of a Pharmaceutical Micro-production Laboratory for antidotes was approved. However, the necessary funds were not allocated and the objective was not achieved. This problem must be resumed in the current political-military context and updated according to the legislation in force. We present below, as an example, some products resulting from the CMMSR’s activity, such as Patents of invention/innovation and/or experimental/galenic models (Table 1). To these can be added numerous sets and diagnostic and treatment kits for medical intervention in CBRN events, some of which have been presented at military exhibitions: ACTTM Clinceni, EXPOMIL Bucharest, BSDA, Inventions fairs, etc. and have been used in applications and theaters of operations (Saudi Arabia, Somalia, Angola, Iraq, etc.).

Regarding orphan drugs, which are very rarely needed, the cost of making and placing a medicinal product on the market for the diagnosis, prevention or treatment of the disease, cannot be recovered through the expected sales of the medicinal product, and the pharmaceutical industry would be refractory to its realization, under normal market conditions. The antidotes for medical protection in case of CBRN attack, terrorism, catastrophe or industrial accident are also considered orphan drugs.

Another aspect of these medicines is represented by the expired drugs. The supplier guarantees the efficacy of the drug only within the deadline, so an orphan drug may expire before it is needed. This can create a financial problem that is difficult to solve. The US Army is currently studying the possibility of extending the shelf life of medicines from stocks, if they have retained their pharmacological properties (internet).

The operating costs differ depending on the chosen variant, the assortments and the quantities of antidotes manufactured, being able to reach the order of millions of euros, depending on the spaces, equipment, personnel and portfolio of pharmaceutical products.

Implementation Opportunities. Providing antidotes is mandatory in medical practice and especially in military medicine. Antidote insurance is nationally deficient and globally they are considered "orphan drugs".

A compromise formula, adapted to the current context in Romania, could be the strategic cooperation regarding fundamental, applied, developmental, innovation scientific research integrated with the production and education of the profile. Thus, the Ministry of National Defence, through virtual reorganization can achieve an Integrated Technological Platform, by sharing the scientific basis and launching the production of antidotes for public health needs and for CBRN crisis situations.

To this would contribute first of all the Military Medical Research Center (CCSMM), the “Cantacuzino” National Military-Medical Institute for Research Development (INCDMM Cantacuzino), the CBRN Defense and Ecology Scientific Research Center (CCSACBRNE), as well as other competent military entities and civilians, from the academic, university, industrial health field, etc. In order to launch the specific antidote micro-production or production, it may be necessary to build or adapt an appropriate facility (pharmaceutical laboratory or drug factory). As a back-up formula, in case of force majeure, a virtual "ad hoc" structure can be created, which will integrate different structures and microstructures existing in Ministry of National Defence, to achieve this common purpose.

Table 1: Examples of CBRN medical protection products resulting from the activity conducted by the Military Medical Research Center

No	Product	
1	Medicinal product with effective action against organophosphorous compounds	Patent State Office for Inventions and Trademarks 79914/1974 V. A. Voicu, C. Miu, M. Jiquidi, C. Furnica, I. Elian
2	Dixidextracaine 70, a drug intended for subarachnoid hyperbaric anestesia	Patent State Office for Inventions and Trademarks 81100/1975 I. Curelaru, C. Furnica, V. A. Voicu, E. Ionescu-Visan, C. Miu, P. Nitescu, L. Ivanescu, I. Manolache
3	Pharmaceutical combinations with antiseptic effects	Patent State Office for Inventions and Trademarks 65237/1977 Al. Bors, V.A.Voicu, C. Rizescu
4	Dynamic repolarization solution	Patent State Office for Inventions and Trademarks 65235/1977 Al. Bors, V .A. Voicu
5	Anti-arrhythmic compound	Patent State Office for Inventions and Trademarks 65236/1977 Al. Bors, V .A. Voicu
6	Equipement for objectifying drug interactions	Patent State Office for Inventions and Trademarks 74803/1980 Al. Ionescu-Stoian, V. A. Voicu, M. Jiquidi
7	Anti-arrhythmic compound and synthesis process	Patent State Office for Inventions and Trademarks 69027/1978 Al. Bors, V. A. Voicu, V. Aldea, E. Olteanu
8	Radioprotective compound	Patent State Office for Inventions and Trademarks 3198/1970 Al. Bors, V. A. Voicu, C. Paun, Gh. Dumitrescu
9	New anti-hypertensive compund	Patent State Office for Inventions and Trademarks 76911/1980 V. A. Voicu, St. Muresan, Gh. Radu, M. Jiquidi
10	New anti-hypertensive compund	Patent State Office for Inventions and Trademarks 76912/1980 V. A. Voicu, St. Muresan, Gh. Radu, M. Jiquidi
11	New antiseptic compound	Patent State Office for Inventions and Trademarks 80051/ 1979 Al. Bors, V. A. Voicu, A. Karassi
12	Anticholinergic compound	Patent State Office for Inventions and Trademarks 87010/1985 V. A. Voicu, Al. Ionescu-Stoian, L. Antoniu, M. Jiquidi
13	Herbal compound with anti-inflammatory, analgesic and vasoprotective action	Patent State Office for Inventions and Trademarks 87147/1985 Al. Ionescu-Stoian, L. Antoniu, V. A. Voicu, V. Florea
14	Complex antidote for the absorption, neutralization and inactivation of ingested toxins	Patent State Office for Inventions and Trademarks 99921/1989 V. Mihaila, V. A. Voicu
15	Medicine with anti-inflammtory, analgesic and decongestant effect	Patent State Office for Inventions and Trademarks 102954/1991 V. A. Voicu, C. Mircioiu, Al. Ionescu-Stoian, M. Jiquidi, E. Rolea
16	Collagen bandage for the treatment of burns	Patent State Office for Inventions and Trademarks 103550/1991 V. A. Voicu, V. Trandafir, T. Giurgiu, T. Safta, D. Burlacu, A. Nica, L. Dragoescu
17	Medicine with anti-inflammtory, analgesic and decongestant effect	Patent State Office for Inventions and Trademarks 106333/1996 V. A. Voicu, M. Jiquidi, C. Mircioiu
18	Method and compounds for the simultaneous determination of acetyl-cholinesterase and pseudo-colinesterase in blood	Patent 67856/19.10.1976
19	Versatile antidote against chemical agents, with effect on skin lesions and mucous membranes	Patent 7959/23.06.1978
20	Device for measuring the caloric response	
21	Protective mixture for impregnating fabrics	
22	Device for measuring contamination with radioactive isotopes of the human body	
23	Device for rapid desiccation of biological or chemo-pharmaceutical compounds	
24	Medicinal product with specific anti-stress, anti-wear synergistics, polyvalent, neuropsycholobiological revitalization effects	Invention Patent No. 87905/2005
25	Immuno-enzymatic test for the detection of microorganisms	Patent 91566/1984/09.01.1987
26	Enzyme conjugation process	Patent 90105/1984
27	Herbal compound with anti-inflammatory, analgesic and vascular-protective effect	Patent 9992/30.10.1989
28	Collagen bandage for skin lessions	Patent 103550/20.02.1989

Military medicine has had and has the conceptual capacity to make antidotes for CBRN medical protection, some of which have been patented, but it does not have the production capacity. It is possible to arrange or build a self-production capacity for specific antidotes, as orphan drugs, on constructive variants, depending on complexity. The total costs are important, but the objective can be amortized in a short time if the supplier has a regional opening and the social benefit is very high.

The problem of self-injection syringes

We consider that the most important and stringent problem is to equip the active and the non-commissioned military staff and the risk group population, with self-injection syringes (over 1 million people in Romania), according to NATO norms (STANAG 2871 CBRN MED, 3-6 syringes per person, plus reserves); therefore, a total of over 3-6 million self-injection syringes.

The issue of providing CBRN antidotes in Romania is not solved by NATO standards. For example, the RDG model self-injection syringes, made of stainless steel, that were in the Romanian Army's endowment, were withdrawn in the 1980s and have not been replaced until now. The Romanian model made of plastics (made by the Center for Military-Medical Scientific Research as an experimental model, a functional experimental model, a prototype and a zero series) was no longer manufactured in series due to the privatization of the manufacturer (the Plastics Factory Bucharest). At present, the Center for Military-Medical Scientific Research has developed a prototype of self-injecting syringe for antidotes, with the intention to carry out a technological transfer through projects funded by the Ministry of Research and innovation.

THE PHARMACEUTICAL LABORATORY

The pharmaceutical laboratory for the production of antidotes for CBRN medical protection must be a basic component of CBRN medical protection. It must be complex, must have functional autonomy (space, equipment, staff and specific procedures) and must operate in compliance with national and international norms in the field. Its main activity shall consist in the pharmaceutical research, manufacture and pharmaceutical control of CBRN medical protection medicines (antidotes and decontaminants), as well as the storage (in pre-determined quantities) and must be in charge of their distribution (in effect of an order) to the military or the civilian health network, nationally or internationally.

The Romanian Army had 17 facilities authorized for the microproduction of military-specific drugs, including antidotes: 12 in the pharmaceutical laboratories of the Military Hospitals, one in the pharmaceutical laboratory at the Bucharest Garrison Health Center (led by chief pharmacist colonels (eg Col. Pharm. Dr. Oiţa from Iasi who prepared and patented over 100 innovative drugs), a laboratory for blood and derivatives at the Hematology Center (led by Col. Pharm. Dr. Coreni), a galenic laboratory for antidotes at Military Medical Research Center (CCSMM) (led by Col. Pharm. Prof. Dr. Mircioiu), one at the Pharmacology and Drug Control Laboratory (led by Col. Vatasescu), a microproduction laboratory for specific drugs for the military staff, at the Sanitary Warehouse (led by Col.Pharm. Rosnovschi), and a chemical synthesis laboratory for antidotes (led by Col. Pharm. Miu) was also operating at CCSMM. These had incorporated a department for injectable and infusible medicines and were able to prepare any master or galenic drugs recommended by clinicians or researchers. However, all were abolished at the restructuring of the army from the beginning of this century, and the current legislation no longer allows them to be re-instated.

We recall that the Medical Directorate had a Pharmaceutical Microproduction Laboratory, with an old tradition, that provided specific sanitary-pharmaceutical materials, but it was abolished, after almost a century of existence, at the entry into force of the Medicines Law, because it did not comply with the requirements imposed by the European Union, although strictly speaking, they refer only to the "marketing authorization" of the products.

It can also be exemplified based on the experience of CCSMM, that had a Galenic Laboratory, which implemented the products created by the researchers from the laboratories of the Center (under direction of General Prof. Victor A. Voicu, MD, PhD, SR, member of Romanian Academy) but which was renamed and reorganized, because the new Medicines Law no longer provides the "galenic" category. This laboratory, with pharmacists and chemists, had the task of pharmaceutical, pharmacological research and prepared the reagents and drugs resulting from scientific research activities of the center, in quantities of up to 50 doses per lot, in order to remain in the field of microproduction. The products were used for physico-chemical tests, animal experiments or cell cultures and some were distributed to units located in Operations Theaters (for example in Iraq, Somalia, Angola), in applications (for example Campulung, Brasov, Tulcea), in specific missions (for example, anthrax, cholera, BDA epidemics), have been presented at different thematic exhibitions (Clinceni, Central Army House, Expomil, Inventions, BSDA) and many have been patented as inventions or innovations. However, at the

present moment, the Romanian Army no longer has any facilities for drug production, so neither specific CBRN antidotes nor qualified personnel for pharmaceutical preparations.

REQUIREMENTS AND LEGISLATION IN FORCE FOR CERTIFICATION/ACCREDITATION/OPERATION

After designing, obtaining the necessary permits and financing, the platform can be built and the specific endowment can be started. From the moment it is put into operation, with the commissioning protocol, the sanitary-veterinary authorization of operation is requested from the Center for Preventive Medicine, then the RENAR and ANMDM approval for the manufacturing authorization. After completing these mandatory steps, it can be proceeded to micro-production, but for each particular product the marketing authorization must be obtained.

Corroborating the need of medical research to physically make the newly designed products (medicines, diagnostic kits, sets and medical devices), at the level of experimental model (EM), functional experimental model (FEM), prototype and/or technological demonstrator, in order to obtain the approval, commissioning protocol, authorisation and possibly patenting (as an invention or industrial model), with the need for the industry to carry out the microproduction and serial production of 0 series for clinical trials and obtaining the marketing authorization (MA), the integrative principle must apply: research, education and production.

The current legislative requirements can be found in: the Guide on good manufacturing practice for medicinal products for human use, the Guide on good practice in drug distribution, the Order 1807/2006 regarding the approval of the Norms for the application of some provisions of Regulation no. 141/2000/EC on orphan drugs, entered into force in 2007, GMP Regulations – Good Practice Regulations in production, consultancy-certification.ro, etc.

CONCLUSIONS

Medical countermeasures for CBRN defense are based on the prophylactic and curative treatment of the infected and the sick. Depending on the rapid and correct etiological diagnosis, the appropriate treatment is immediately implemented. However, in time of peace, this case is almost non-existent, and during the war or during a terrorist attack it can be a mass pathology, like an explosive epidemic, in which the forces and means involved for medical counter-measures become insufficient.

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10. *** Legea medicamentului
11. *** Ordine ale Ministrului Sănătăţii
12. *** Ordinul 1807/2006 privind aprobarea Normelor pentru aplicarea unor prevederi ale Regulamentului nr. 141/2000/CE privind medicamentele orfane, intrat în vigoare în 2007
13. *** Reglementări ANMDM
14. *** Regulamentele GMP - Reglementări de Bună Practică în producție, consultanță-certificare.ro
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16. *** STANAG 2871 CBRN MED
17. *** WHO Model List of Essential Medicines, Ediția 20, OMS 2017

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ORIGINAL ARTICLES

Non-melanoma skin cancer (NMSC): Extramammary Paget ’s disease

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Abstract: *Non-melanoma skin cancer (NMSC) comprises squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and severalrare skin tumors (i.e., Extramammary Paget’s disease), being the most common malignancy-affecting people worldwide. Itrepresents the majority of skin cancers and a significant percentage of all malignancies. Despite increasing public awarenessand scientific interest, the incidence of NMSC is constantly increasing. Even though most NMSC`s are associated with lessaggressive behavior, they can still be invasive locally and cause extensive damage to neighboring structures, inducingsignificant morbidity. In addition, different types and subtypes of NMSC tend to have frequent recurrences and may have significantmetastatic potential. As a direct consequence, NMSC has become an important issue for healthcare systems with a significantsocio-economic impact.*

Keywords: *Extramammary Paget’s disease; World Health Organization; keratinocyte stem cells; EMA*

INTRODUCTION & BACKGROUND

Non-melanoma skin cancer (NMSC) comprises squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and several rare skin tumors (i. e. Extramammary Paget’s disease), being the most common malignancy affecting people worldwide. It represents the majority of skin cancers and a significant percentage of all malignancies. Despite increasing public awareness and scientific interest, the incidence of NMSC is constantly increasing. Even though most NMSC`s are associated with less aggressive behavior, they can still be invasive locally and cause extensive damage to neighboring structures, inducing significant morbidity. Also, different types and subtypes of NMSC tend to have frequent recurrences and may have significant metastatic potential. As a direct consequence, NMSC has become an important issue for healthcare systems with a significant socio-economic impact.

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EXTRAMAMMARY PAGET’S DISEASE (EMPD)

EMPD localized to the vulva is a rare disease that canmimic different types of dermatosis and only four cases havebeen reported in our practice in the last 5 years.

Definition

EMPD, according to World Health Organization, is a rare intraepithelial neoplasm characterized histopathologically by a prominent growth of large cells, with apocrine or eccrine glandular-like pattern, originating in the skin (primary EMPD) or representing intraepithelial spread of a hidden visceral carcinoma (secondary EMPD). This type of disease is represented by the International Classification of Diseases for Oncology with the following code: 8542/3. [1, 2]

EMPD is a marginated plaque that clinically and histologically

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resembles Paget's disease, but occurs in sites rich in apocrine glands, such as vulva, anogenital region and axilla. Currently, there is controversy as to how often this condition appears against on the background of an underlying carcinoma and how often it occurs primarily in the epidermis or apocrine ductal tissue in the affected area. This gave rise to the concept of primary and secondary extra-mammary Paget's disease [3, 4].

Epidemiology and Etiology

EMPD accounts for about 1 % of all ano-genital neoplasms. It develops more frequently in Caucasian women in the postmenopausal period [1] and usually begins in the fifth decade or later. Current opinion is that, in about 75% of cases, extracellular Paget's disease appears as a primary intra-epidermal neoplasm, possibly from ductal cells of the apocrine gland or keratinocyte stem cells. Another possible origin may be the Toker's cells [3, 5]. In the remaining 25% of cases, there is a hidden primary adenocarcinoma. These cases are referred as secondary Paget's disease.

Only a few cases of association between mammary and vulvar Paget's disease have been described. Under extraordinary circumstances, EMPD is a secondary event caused by extension of an adenocarcinoma either of the rectum to the perianal region, or of the cervix to the vulvar region. On the other hand, genital Paget's disease with a long evolution, can extend into the cervix and urinary tract [6].

Localization

The labia majora is the most widespread site, followed by the labia minora and clitoris. The perineum, perianal area, scrotum, penis, lower abdomen and inguinal folds can also be affected [1].

Based on origin, several classification schemes have been proposed [22] :
Type 1: primary vulvar cutaneous origin
Type 2: secondary to primary anal or rectal adenocarcinoma
Type 3: secondary to urothelial carcinoma

OR

Type I: Paget disease of primary cutaneous origin
Ia: as a primary intraepithelial neoplasm
Ib: as an intraepithelial neoplasm with invasion
Ic: as a manifestation of underlying adenocarcinoma of skin appendage or vulval glandular origin
Type II: Paget disease of noncutaneous origin
IIa: secondary to anorectal carcinoma
IIb: secondary to urothelial neoplasia
IIc: as a manifestation of another noncutaneous carcinoma: endocervical, endometrial or ovarian adenocarcinoma, etc.

Clinical features

The most common presentation is a relatively well-demarcated flat or slightly elevated, scaly, erythematous patch or plaque, accompanied by itching or burning, often simulating eczema. Unusual forms include lesions with hyper- or hypopigmentation and multi-centric bruising [1].

EMPD lesion has many features in common with Paget's disease of the nipple. The edges are sharp, rounded and slightly raised, and includes an area that is pink or red. The affected area may be tiled, and small, gray-colored crusts may cover erosion. Itching is an essential feature and there may be excoriations or lichenification. Areas of fluctuating hyperpigmentation may be present, causing confusion between extra-mammary Paget's disease and superficial spread melanoma. In some cases, leukoplakia may be present [3, 7, 8, 9].

The clinical presentation of the disease varies by site, the most frequent involved being vulva. The first symptom, especially in vulvar lesions, is itching and burning, which can be persistent and growing. It is often seen as dermatitis and may be disturbed by topical therapy. The surfaces of the lining of the labia are periodically redder than the skin when both areas are involved, and the change may proliferate towards the thighs, mons pubis and vaginal introitus. There will be a limited papillomatous area. Characteristic clinical features include fierce advancement, challenging all local applications, and the whetted margin. Eventually, one area may become thickened and ulcerated as evidence of downwards invasion. Lymph nodes involvement or distant metastases can appear. Although most cases of primary carcinoma result from a hidden adenocarcinoma of the sweat gland, it is essential to examine the patient for evidence of a later adenocarcinoma hidden in the cervix and rectum [3].

Pathogenesis

Histogenesis of primary EMPD is unknown [1].

Paget cells in the epidermis may appear as an in situ upward extension of an adenocarcinoma located in the deeper glands. Alternatively, EMPD may have a multifocal primary origin in the epidermis and skin appendages, and does not represent an epidermotropic spread or metastasis from an intrinsic carcinoma of the sweat gland. It can be shown that almost 25% of EMPD originates from primary adenocarcinoma of the secretory glands. Primary tumor and Paget cells are usually mucus secretory [10].

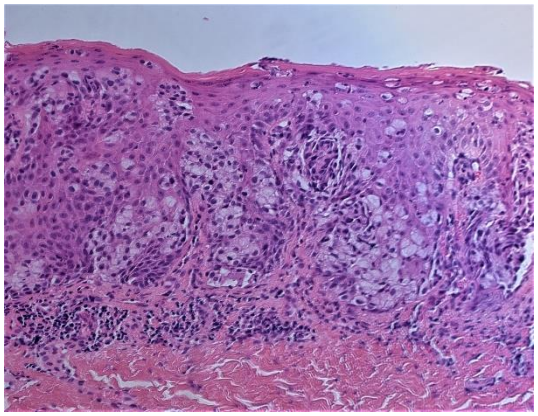
At one point, it was widely accepted that in primary EMPD, Paget cells in the epidermis are due to an in-situ upward extension of an in-situ adenocarcinoma of the sweat glands

along the eccrine or apocrine ducts. This theory is comparable to the generally accepted view that secondary EMPD shows an extension of a cervical, rectal or urinary adenocarcinoma and that the progression of Paget breast disease is an extension of a lactiferous canalicular epidermotropic carcinoma. The fact that a hidden adenocarcinoma often could not be demonstrated, can be explained by the different anatomic characteristics between the breast, which has only 20 large, conspicuous lactiferous ducts, and the genital skin, which has thousands of small apocrine and eccrine glands, making the localization of this particular small gland invaded by carcinoma increasingly difficult [6].

Histopathology

EMPD disease has in common with Paget's disease the presence of varying numbers of Paget cells within the epidermis (Figure 1). In some cases, the Paget cells are found to be limited to the acanthotic epidermis, accompanied by hyperkeratosis, parakeratosis, erosion or ulceration. Frequently, Paget cells can be identified within the epithelium of hair follicles or eccrine sweat ducts. This types of in situ malignancy associated with EMPD has the same favorable prognosis as EMPD limited to the epidermis. However, the prognosis is much worse in cases in which the Paget cells have invaded the dermis from the epidermis or from an underlying sweat gland carcinoma. Glandular clusters with a central lumen, absent in mammary Paget's disease may be identified in the lower epidermis in extra-mammary Paget's disease [6].

Figure 1: EMPD. Paget cells in epidermis (photomicrograph taken from our cases), hematoxylin &eosin, magnification 200x



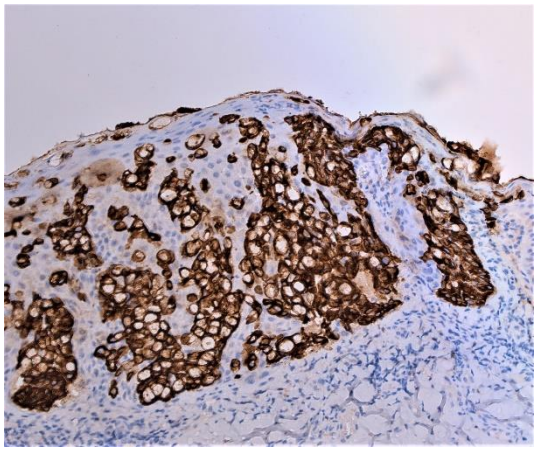
Serial sectioning of excised lesions of vulvar EMPD demonstrated that the lesions within the epidermis and its appendages have a multifocal origin. In the cases that have extensive epidermal lesions, the involvement of eccrine glands and ducts is occasionally, and the involvement of apocrine glands and ducts is absent, even if some Paget cells form apocrine glandular structures in the epidermis. Despite the existence of cohesion between the epidermal and the ductal foci, no one can definitive say that the extension is in an up or down direction. The dermal invasion in EMPD has its beginning point in the epidermis and not ductal or glandular structures.

The origin of the Paget cell may be the poral portion of an apocrine duct, or the pluripotential germinative cells within the epidermis [6]. Paget cells have an abundant basophilic to amphophilic cytoplasm and vesicular nuclei with pronounced nucleoli. They are generally distributed in a solitary fashion or may form gland-like structures or well-demarcated solid nests. Involvement of adnexa (hair follicles and eccrine ducts) is seen in most cases. Rarely, single cells, glands and solid nests can be found in the dermis; this is considered to be microinvasion. Dermal invasion is rare in vulvar EMPD, more common in perianal region. However, this type of microinvasion cannot be applied in cases that are associated with an underlying adenocarcinoma with a ductal in-situ component [1].

Immunohistochemistry

Immunohistochemical staining shows that Paget cells stain consistently positive for CK7 (cytokeratin 7) [1] (Figure 2) and EMA (Epithelial membrane antigen) – used by pathologists like a marker of Paget disease [11] (Figure 3).

Figure 2: Paget cells showed positivity for CK7 (cytokeratin 7also called as keratin 7, CK7, KRT 7, K7) (photomicrograph taken from our cases), magnification 200x



Paget cells often express GCDF-P15 (gross cystic disease fluid protein) (Figure 4), ERBB2 (HER2/neu) (Figure 5), CEA (carcynoembrionic antigen), CA125 (cancer antigen 125/ MUC16), and AR (androgen receptor) [1].

Figure 3: Paget cells showed positivity for EMA (epithelial membrane antigen also known CD227, MUC1 or episialin) (photomicrograph taken from our cases), magnification 200x

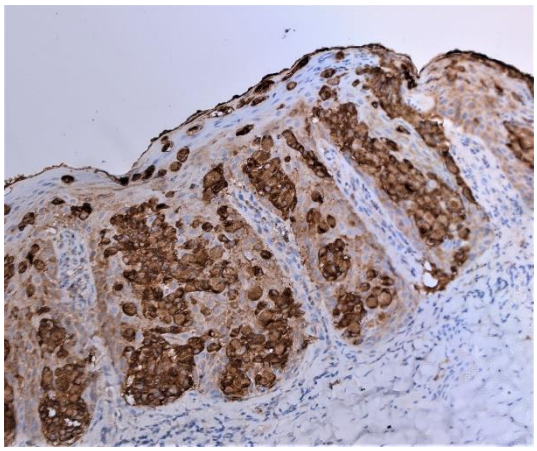


Figure 4: Paget cells showed positivity for GCDFP-15 (gross cystic disease fluid protein also known BRST2, PIP-prolactin induced protein) (a photomicrograph taken from our cases), magnification 200x

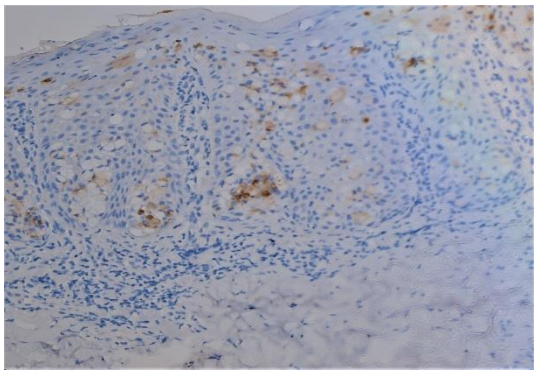
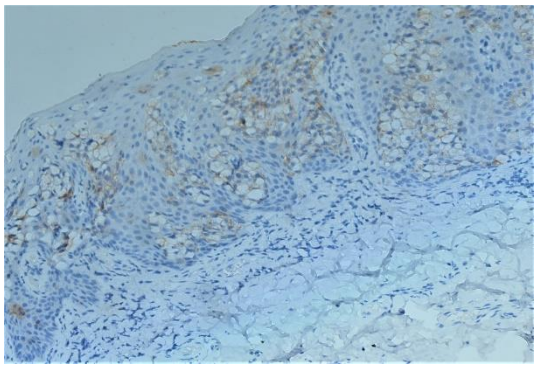


Figure 5: Paget cells showed positivity for ERBB2 (human epidermal growth factor receptor 2, also known HER2/neu, c-erbB2, CD340)(photomicrograph taken from our cases), magnification 200x



Differential diagnosis.

For correct diagnosis, immunohistochemistry and clinico-pathological correlation are essential [1].

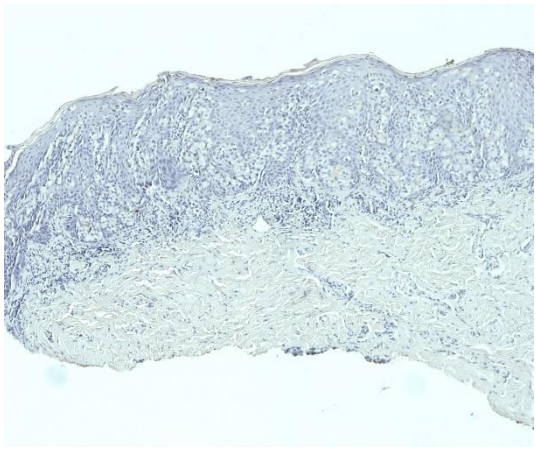
The most pressing matter is distinction between primary and secondary EMPD (Table 1). Potential imitators of EMPD are Toker vulvar cells, vulvar and penile analogues of cervical stratified mucin-producing intraepithelial lesion, high-grade squamous intraepithelial lesion, melanoma, pagetoid dyskeratosis, and clear cell papulosis. [1]

Table 1: Differential diagnoses of Extra-mammary Paget's disease (Immunohistochemical Markers). [12]

Immuno-histochemical marker	Primary extra-mammary Paget's disease	Secondary extra-mammary Paget's disease	Bowen's disease	Superficial spreading melanoma
CK7	+	+	+ or -	-
EMA	+	+		
CEA	+	+	-	-
GCDFP-15	+	-	-	-
Melan-A	-	-	-	+
P63	-	-	+	
S100 protein	-	-	-	+
HMB45	-	-	-	+

For differential diagnosis with superficial spreading melanoma we currently use as immunohistochemical markers S100 protein and HMB45 (Figure 6), and we do not use Melan-A because it has often given false positive results (Figure 7).

Figure 6: Paget cells showed negativity for HMB45 (Human Melanoma Black) (photomicrograph taken from our cases), magnification 100x



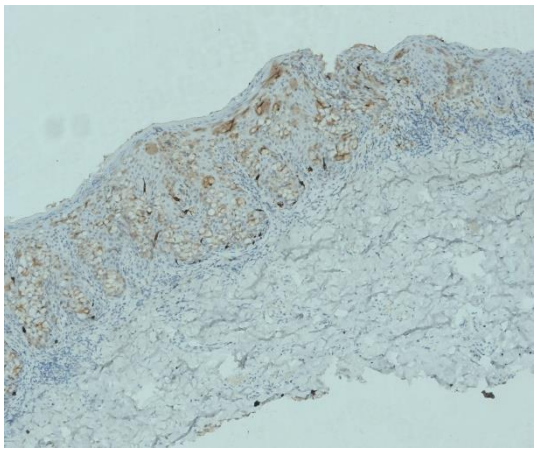
For proper localization of a large perineal lesion (vulvar or perianal) we usually use GCDFP-15 (positive for vulvar localization) and CK20 (positive for perianal localization) [13]

Prognosis and treatment

Primary EMPD's prognosis generally is better than that of mammary Paget's disease [6].

Local recurrence is frequent, even in cases with a wide surgical extraction, the recurrence rate being 12-58%. This may be related to positive surgical margins, skip areas, and multicentricity.

Figure 7: Paget cells showed false positivity for Melan-A (Melanoma Antigen, also called MART-1 – Melanoma Antigen Recognized by T cells 1)(photomicrograph taken from our cases), magnification 100x



Adverse prognosis is associated with increased serum levels of CEA and the depth of invasion. Proper tissue sampling and other investigations are vital to determine if there is an associated underlying malignancy that requires surgical excision. If such a malignancy exists, it should be excised along with all clinically abnormal epithelium. If no underlying malignancy is clinically and surgically detected on careful examination, the entire affected area of skin should be excised.

In the case of primary EMPD associated with an invasive component, the prognosis is significantly worse, and it can undergo lymph node metastasis. Cases with gene mutation of PIK3CA and AKT1 are often invasive. [1]

An important method is "Mohs surgery" (microscopically controlled surgery) that minimizes the recurrence rate by carefully controlling excision margins of the tumor [14, 15, 16].

Photodynamic therapy is another important tool in the treatment of EMPD, but it requires larger series and longer periods of follow-up [17, 18].

Topical imiquimod has been used with good results in cases with limited disease [19].

Radiation therapy has been supported either as an adjuvant to surgical therapy [20] or as an alternative therapy for elderly patients in whom surgery may have a greater risk [21].

DISCUSSIONS

Because of the resemblance of the lesions in the EMPD with other dermatological conditions, it is important that the clinician always consider a possible diagnosis of EMPD, especially in the case of lesions in the genital and anal spheres.

Due to the false positive results that may appear at various markers used for the diagnosis of EMPD, the pathologist cannot always rely on immunohistochemical tests for differential diagnosis with other disorders. Therefore, a correlation between clinical, paraclinical and histopathological data is very important for a correct diagnosis.

CONCLUSIONS

EMPD is a very rare skin malignancy that needs to be diagnosed and treated accordingly [23]. Because it can mimic a lot of benign or malignant diseases, a correct differential diagnosis is crucial for the treatment and prognosis of the patient. Also, it is imperative to differentiate primary EMPD from a secondary type, in the latest the identification of the primary site of the tumor being vital for the patient.

Author Contributions: All authors had equal contribution in this study elaboration.

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

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ORIGINAL ARTICLES

Laparoscopic sleeve gastrectomy and esophageal motility

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Abstract: *Laparoscopic sleeve gastrectomy (LSG) is becoming popular between the bariatric procedures. There are few studies available in the literature about LSG impact on esophageal motility, with debatable results. Technically, it modifies the anatomy of the esophago-gastric junction; it can damage the phreno-esophageal ligament and decrease the lower esophageal sphincter pressure that will predispose to reflux. Gastroesophageal reflux disease (GERD) is already common in obese patients due to frequent hiatal hernia, high intraabdominal pressure, increased transient lower esophageal sphincter relaxations (TLESR), altered esophageal motility and poor esophageal clearance. LSG can worsen gastroesophageal reflux disease, but also the weight loss achieved after it and the decrease of acid production in the small gastric pouch will probably reduce the GERD symptoms. Dysphagia and odynophagia were reported after sleeve gastrectomy, but usually associated with anatomical narrowing of the gastric sleeve, caused by esophageal dismotility or secondary to diabetes mellitus, low tiamine level, the use of opioids and non-steroidal anti-inflammatory drugs, even hypothyroidism. In addition to upper GI tract endoscopy, high-resolution esophageal manometry (HRM) should be required for all patients planning to undergo a LSG, and in the follow-up period according to the contractility pattern.*

Keywords: *obese, sleeve gastrectomy, esophageal motility, esophageal clearance, reflux*

INTRODUCTION

According to World Health Organization (WHO), overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. If the Body Mass Index (BMI weight in kilograms divided by the height in square meters) is between 25-30 kg/m^2 a person is overweight, and if BMI > 30 kg/m^2 obese [1]. Unfortunately due to behavioral and nutritional changes more than 20% of the worldwide population is overweight and 10% obese, and in the next 20 years the estimation is that 2.16 billion people will be overweight and 1.12 billion obese [2].

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Morbid obesity, defined by BMI greater than 40 kg/m^2 or between 35 and 40 kg/m^2 with any obesity-associated conditions such as diabetes, high blood pressure, cardiovascular disease, stroke, sleep apnea, osteoarthritis, is a serious invalidating health condition that can interfere with basic physical functions such as breathing or walking [3].

The first step to obtain weight loss is through behavioral and lifestyle changes, with low-calorie diet and exercise, but usually previous attempts of weight loss are followed by rapid weight regain. Many obese associate to diet and exercise pharmacological drug therapy, achieving maximum 10% weight loss [5]. Unfortunately, a lot of patients fail to achieve long-term weight loss through these methods, so they are good candidates for surgical treatment.

Bariatric surgery cause weight loss by restricting the amount

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of food in the stomach, or by a combination of gastric restriction and malabsorption[6]. The bariatric surgical procedures can be purely restrictive, such as laparoscopic adjustable gastric band (LAGB) or laparoscopic sleeve gastrectomy (LSG), or malabsorptive-restrictive: Roux-en-Y gastric bypass (RYGB) and biliopancreatic diversion (BPD)[3, 6]. All the bariatric surgical procedures may have local complications (stenosis at anastomotic sites, stomal ulcers, band erosion, fistulae) but they can be managed with proper follow-up and close monitoring.

Table 1: The known risk factors [4] for morbid obesity are:

1. Genetic factors (familyhistory of obesity)
2. Dietaryhabitsandsedentary lifestyle, **q**uittingsmoking, microbiom
3. Stressandanxiety (highlevel of cortisol, high-calorie food)
4. Sleepdisorders
5. Female (post pregnancyweightgain or menopause)
6. Aging (slowingdown metabolism)
7. Somemedications (antidepressants, steroidsand beta-blockers)
8. Cushing'ssyndrome, Prader-Willi syndrome

The laparoscopic adjustable gastric band (LAGB) involves an inflatable band that is placed around the upper portion of the stomach, creating a small gastric pouch and reducing the amount of food. Is a reversible procedure, with shorter hospital stay and low mortality rate, but the band can slip or create erosion into the stomach, requires a foreign device to remain in the body and strict adherence to the postoperative diet and follow-up visits. The weight loss achieved is slower than the other bariatric procedures, with low risk of vitamin/mineral deficiencies, but a high rate of re-operation [6].

The Roux-en-Y gastric bypass was considered the "gold standard" of weight loss surgery, restricting the amount of food consumed and modifying hormonal level of ghrelin, decreasing appetite an enhance satiety. But is technically more complex than LAGB or laparoscopic sleeve gastrectomy (LSG), has longer hospital stay with possible long-term vitamin/mineral deficiencies (vitamin B12, calcium, folate, iron). Nowadays, remains an effective method to alleviate symptoms of GERD and many bariatric procedures are finally converted to Roux-en-Y bypass [6].

Laparoscopic sleeve gastrectomy (LSG) consist of a vertical division on the larger curvature of the stomach, removing a large portion of the body and all of the fundus, the effect being both restrictive and endocrinologic – reducing ghrelin. LSG is very popular among the bariatric procedures, it induces rapid and sustainable weight loss of > 50% for 3-5 years, has relatively short hospital stay (48h), but is a non-reversable procedure with potential long-term vitamin deficiencies [6]. Also, transforming the stomach into a cylindrical structure and altering the anatomy of the esophago-gastric junction will probably influence the lower esophageal sphincter pressure and esophageal motility.

OBESITY AND ESOPHAGEAL HEALTH

Gastroesophageal reflux disease (GERD) is common in obese patients, and they can be even more sensitive to the presence of acid in the esophagus than a normal weight person. Also, hiatal hernia, a risk factor predisposing to GERD is more prevalent among the obese [7]. Obesity may trigger vagal abnormalities and a higher level of bile and pancreatic enzymes, worsening the effect on esophageal mucosa [8].

According to a meta-analysis published by Halmpel H. et al. in 2005 [9], obesity may increase the risk of GERD symptoms by 50%, and also cause a 2-fold increase in esophageal adenocarcinoma [9]. The pathophysiological mechanisms of increased GERD in obese are: high intraabdominal pressure, frequent hiatal hernia, increased transient lower esophageal sphincter relaxations (TLESR), lower esophageal sphincter (LES) pressure, altered esophageal motility with poor esophageal clearance [10, 11].

The esophageal transit time in obese has been in the researchers preoccupation from1985, when Mercer CD et. al [11] measured it by radionuclide scintigraphy using a swallowed technetium sulfur colloid bolus. The data obtained were compared from obese patients with GERD to normal weight with and without GERD. The esophageal transit time was significantly prolonged in the obese group compared with both lean groups (p less than 0.001). Also performing conventional esophageal manometry theyconfirmed that in obese patients gastroesophageal pressure gradient is elevated [12].

Furthermore, in a study published in Obese Surgery in 1999, Jaffin BW et al. revealed a high prevalence of asymptomatic esophageal motility disorders among morbidly obese patients. During a 3-year period, they evaluated manometric and completed a questionnaire of symptoms, medications, demographic data, other medical conditions associated, 111 obese patients for bariatric surgery. Abnormal manometric findings were observed in 61% patients: 14% hypertensive contractions of the esophageal body, 7% diffuse esophageal spasm (DES), 1% achalasia, 25% hypotensive lower esophageal sphincter defined by LES pressure < 10 mm Hg, and 14% were nonspecific esophageal motility disorders [13].

Similar results have been obtained by Koppman's team [14], after performing esophageal manometric evaluation in 116

obese patients selected for laparoscopic gastric banding in 2003. 41% of the patients had abnormal manometric findings: 23% nonspecific esophageal motility disorders, 11% hypertensive contractions, 1% diffuse esophageal spasm, 1% achalasia, 3% isolated hypertensive LES pressure (>35 mmHg), 3% isolated hypotensive LES pressure (<12 mmHg). Only one patient with abnormal esophageal motility reported noncardiac chest pain [14].

LAPAROSCOPIC SLEEVE GASTRECTOMY AND ESOPHAGEAL MOTILITY

In a review of the impact of laparoscopic sleeve gastrectomy on gastrointestinal motility, published in 2018 by Eleni Sioka et al. [15], after a search of the medical literature with two independent reviewers, they selected only 9 eligible studies that focused on esophageal motility [16]. The esophageal motility data were debatable, with differences regarding the follow-up timing (from 6 weeks to median 13 months), the study design, the surgical technique, the bougie size, and the distance from pylorus. To clarify this subject we need more data from future prospective studies on larger cohorts.

Del Genio G et al. [17] studied the impact of LSG on esophageal physiology, performing high-resolution impedance manometry (HRiM) combined 24-h pH and multichannel intraluminal impedance (MII-pH) before and after the procedure, in 25 patiens. After a median follow-up of 13 months, HRiM showed increased ineffective peristalsis and incomplete bolus transit, but normal LES function.

In a retrospective analysis of 53 cases of HRiM performed after LSG, Mion F. et al. [18] found in 77% of patients an increased intragastric pressure (IIGP), even if they were asymptomatic with no specific manometric or impedance changes, and this could be assimilated as a manometric marker of LSG.

An interesting study of Petersen WV et al. [19] published in 2012, proved that LSG increased lower esophageal pressure independent of weight loss achieved after the procedure, thus protecting obese patients from gastroesophageal reflux.

Dysphagia was reported after laparoscopic sleeve gastrectomy, but usually associated with narrowing of the gastric sleeve which means inability to pass a 9.6 mm gastroscope due to stenosis or a sharp angulation [19]. In a study published by A. Nath et al. in september 2016, from 352 patients included, 22.7 % had dysphagia, 9.3% narrow sleeve, 7.1 % sharp angulation, 2.3% stenosis, and they responded well to ballon dilatation (1-3 sessions). Other medical conditions were associated with post-operative dysphagia: esophageal reflux, diabetes, low tiamine level, opioids and non-steroidal anti-inflammatory drugs or hypothyroidism, so dysphagia is not a specific esophageal motility disorder sign [20].

PRACTICAL CONSIDERATIONS

We performed a prospective study in Carol Davila University Central Emergency Military Hospital to determine the manometric pattern of obese patients, the prevalence of esophageal motility disorders prior and after gastric sleeve and the upper GI complications post sleeve.

The aim was to do high-resolution esophageal manometry (HRM) to all obese patients proposed for LSG, before and 6 to 12 months after the procedure, plus upper GI tract endoscopy and GERD questionnaire (GERD-HRLQ). Starting from January 2017, we included in the study 61 morbid obese patients evaluated both endoscopically and manometric. The exclusion criteria were: previous gastric surgery, another bariatric procedure surgical or endoscopically, the use of medications that could affect esophageal contractility or lower esophageal sphincter pressure (prokinetics, calcium-blockers, nitrates). Half of the patients are now in the post-operative evaluation period, because of surgical intervention timing, and the final results will be later published.

Esophageal manometry was performed with a 36-channel High-Resolution Solid State Catheter from Sierra-Given, after 6 h of fasting and previous upper GI tract endoscopy evaluation. The manometric data were obtained through 10 single test swallows of 5 ml of water. The HRM results were then analyzed according to the Chicago Classification v3.0 of esophageal motility disorders, using the ManoView ESO software program (component of the ManoScan system), providing visual images and quantitative physiological parameters.

The principal manometric variables analyzed [21] were summarized in the Table 2.

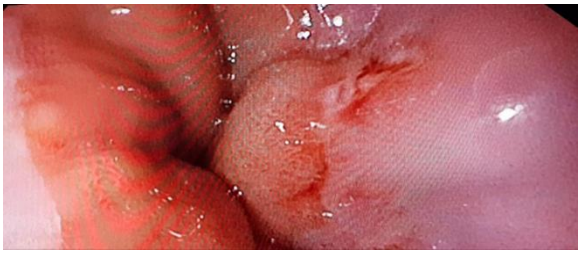
Diffuse esophageal spasm (DES) causes chest pain and sometimes mixed dysphagia for both liquids and solids. The substernal chest pain may waken the patient from sleep or is associated with exercise and often confused with angina pectoris. Hot or cold liquids may aggravate the pain. From the current data available, it is possible that in time this disorder to evolve into achalasia with impaired esophageal peristalsis and lack of lower esophageal sphincter relaxation during swallowing. Usually barium swallow may show poor progression of bolus plus simultaneous contractions [22]. Fragmented peristalsis and ineffective motility are minor esophageal motility disorders according to Chicago Classification v3.0 which can be seen also in normal person.

A particular manometric pattern was found during our study, in a 42-year-old female with morbid obesity BMI = 44.81 kg/m^2 (W= 122 kg, H= 165 cm), obstructive sleep apnea and minor GERD symptoms prior to the surgical intervention. The upper gastrointestinal endoscopy revealed erosive esophagitis grade A, duodenitis with Helicobacter pylori infection both treated preoperative.

Table 2: Principal manometric variables analyzed

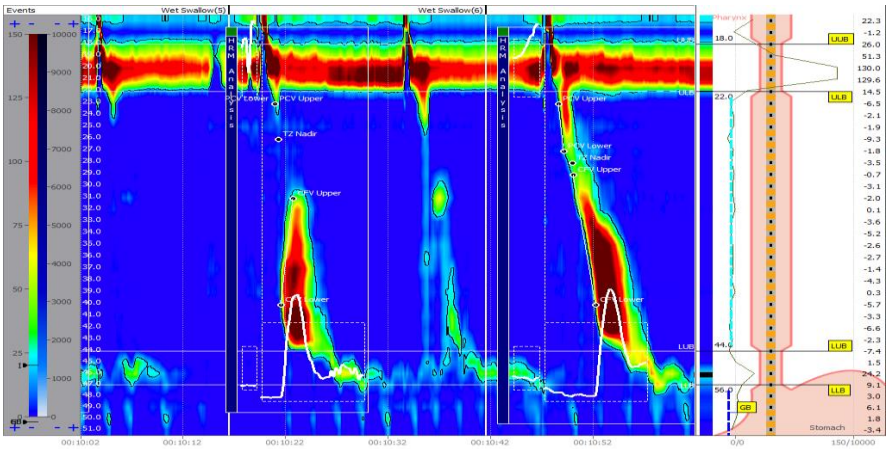
Lower esophageal sphincter(LES) resting pressure	Normal 13-43 mmHg
Deglutitive LES relaxation = Integrated Relaxation Pressure (IRP)	For Sierra-Given solid probe the normal value < 15 mmHg
Esophageal peristalsis = Distal Contractile Integral(DCI)	Normal 500 – 5000 mmHg-cm-sec; > 5000 Hypertensive > 50% contractions weak (DCI < 500) or failed (DCI < 100) = IEM Ineffective motility
Duration of contraction waves in seconds= Distal Latency (DL)	Normal value > 4.5 sec, if 2 contractions are premature < 4.5 sec the diagnose is Diffuse esophageal spasm(DES)
Peristaltic break > 5 cm	More than 50% of swallows = Fragmented peristalsis(FP)

Figure 1: The upper GI tract endoscopy image preoperative – erosive esophagitis grade A Los Angeles Classification



On the preoperative high-resolution esophageal manometry (HRM) she had normal lower esophageal sphincter (LES) basal pressure, LES length = 3 cm, normal deglutitive LES relaxation, with IRP median value of 6.6 mmHg, good esophageal contractility (DCI median= 956.1 mmHg-cm-sec and DCI maximum=1305.7 mmHg-cm-sec) but > 20% premature contraction (distal latency value < 4.5 seconds), aspect of diffuse esophageal spasm according to Chicago Classification v3.0.

Figure 2: Wet 5-ml test swallows recorded with HRM preoperatory – shorten distal latency



Considering the high cardio-vascular risk in a young patient, severe obstructive sleep apnea, the failure of diet and exercise and the need for sustainable weight loss, we didn't contraindicated the laparoscopic sleeve gastrectomy, but we've decided to closely follow the patient.

The laparoscopic surgical intervention went well, with good recovery after the procedure, good tolerance of food intake implementing the diet and exercise protocol. One year after LSG, the patient lost 40 kg, achieved a BMI of 30 kg/m^2 and had 82 kg weight. Clinically, now she had mild dysphagia, early satiety and fullness (that can be related to the low volume of the stomach), and less reflux symptoms compared to baseline.

The result of upper gastrointestinal endoscopy was encouraging: no evidence of erosive esophagitis, a normal gastric scar on the posterior wall of the stomach after LSG, as you can see in Figure 3.

Postoperative data from high-resolution esophageal manometry (HRM) examination revealed normal lower esophageal sphincter basal pressure (mean 20.2 mmHg, min -0.8 mmHg) with normal deglutitive relaxation of LES > 60%, mean IRP value 2.2 mmHg, the same LES length of 3 cm, but more than 50% of the esophageal contractions ineffective, with a DCI < 500 mmHg-cm-sec and normal distal latency,

diagnosis of IEM ineffective motility according to Chicago Classification. Increased intragastric pressure (IIGP) after 5-ml water swallows was observed in 30% of the ten swallows examined.

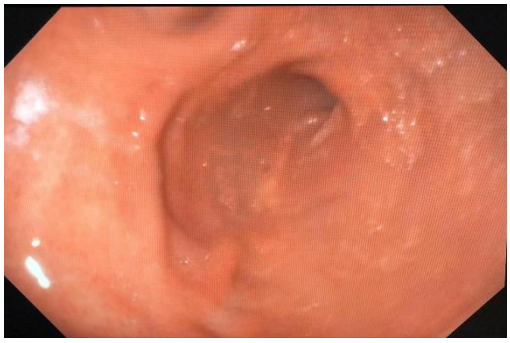


Figure 3: The longitudinal scar on the posterior wall of the stomach after LSG

Figure 4: Wet 5-ml test swallows recorded with HRM postoperatory – ineffective motility

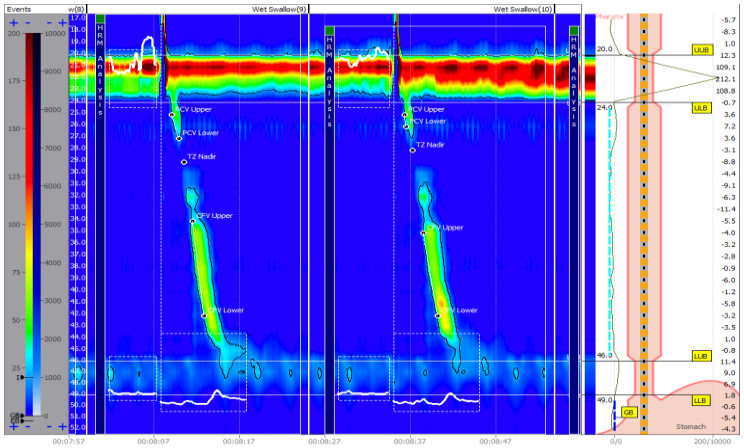


Table 3: The manometric data of LES 1-year after the LSG, no criteria of SED or achalasia as expected

Pressure meas. method	eSleeve	Normal
Basal (respiratory min.)(mmHg)	– 0.8	4.8-32.0
Basal (respiratory mean)(mmHg)	20.2	13-43
Residual IRP (mean)(mmHg)	2.4	<15.0
Residual (highest)(mmHg)	17.9	
Percent relaxation(%)	91	

Table 4: The manometric data of distal esophageal contractility 1-year after LSG (low mean value of DCI = hipocontractility)

High Resolution Parameters		Normal
Distal contractile integral(mean)(mmHg-cm-s)	444.4	500-5000
Distal contractile integral(highest)(mmHg-cm-s)	666.7	
Contractile front velocity(cm/s)	2.9	<9
Intrabolus pressure (@LESR)(mmHg)	– 0.5	<8.4
Intrabolus pressure (avg max)(mmHg)	3.4	<17

Table 5: Detail of all 10 wet test swallows recorded 1-year post LSG (3 failed with DCI < 100 mmHg-cm-sec, 4 weak DCI < 500 mmHg-cm-sec).

	N	Med	1	2	3	4	5	6	7	8	9	10
DCI distal contractil integral (mmHg-cm-sec)	500-5000	444.4	666.7	N/A	422	182.6	N/A	375.7	557	515.2	391.3	N/A

Our patient experienced dysphagia after laparoscopic sleeve gastrectomy, but the GERD symptoms ameliorate. The HRM showed ineffective esophageal motility (IEM) and not achalasia as expected [16]. IEM is considered a minor motility disorder which can be seen also in asymptomatic patients according to Chicago Classification v3.0, but it was also cited by Del Genio G et al. after LSG [17]. We found 30% increased intragastric pressure (IIGP) after water swallows, corresponding to Mion F et al. data [18]. Further manometric follow-up is needed in this patient to see the contractility evolution pattern.

CONCLUSIONS

High resolution manometry (HRM) was already indicated for the preoperative assessment of peristaltic function in patients who needed antireflux surgery, but we consider that it is necessary to extend the HRM recommendation to bariatric surgery for better management of complications.

Studying the manometric changes in obese patients prior to laparoscopic sleeve gastrectomy is important, because it helps to select those patients who could develop postoperative esophageal motor complications and refractory reflux. In addition to upper GI tract endoscopy, high resolution esophageal manometry (HRM) should be required for all patients planning to undergo a LSG.

A proposed alternative to pharmacological treatment to relieve post laparoscopic sleeve gastrectomy GI symptoms is usually the Roux-en-Y gastric bypass, which is considered an effective method to alleviate symptoms of GERD, but it will not resolve the poor esophageal contractility. In conclusion, conversion to gastric bypass is not an universal method for managing GI complications, especially esophageal dysmotility after LSG, so we need to choose wisely the patient eligible for sleeve.

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CLINICAL PRACTICE

Bilateral pneumatization of middle and superior nasal turbinates

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Abstract: *The pneumatization of all nasal turbinates is an extremely rare finding. Several previous reports have presented the bilateral superior, middle, and inferior conchæbullosæ. We report the bilateral pneumatization of middle and superior nasal turbinates. The variant was documented in cone beam computed tomography (CBCT) in all three dimensions. The inferior concha bullosa was a bilaterally moderate subtype of lamellar pneumatization that presented as a conchal recess of the maxillary sinus. The concha bullosa media was bilaterally of the extensive or true type and associated unilaterally with an uncinate bulla. The concha bullosa superior was asymmetrical bilaterally, narrower on the right side, and larger on the left where it was also septated and tri-cameral. The supreme conchæ were rudimentary, as were their pneumatizations. For an accurate anatomic diagnosis of conchæbullosæ in computed tomography, sagittal, coronal, and axial cuts, as well as three-dimensional reconstructions, should be used to ensure adequate planning of functional endoscopic sinus surgery.*

Keywords: *agger nasi; ethmoid bone; nasal fossa; nasal concha; nasal turbinate; anatomic variation*

INTRODUCTION

The lateral nasal wall attaches the nasal turbinates or conchæ. The inferior one is an independent bone. The middle, the superior, and the inconstant Santorini's supreme turbinates are the ethmoidal turbinates that are attached on the nasal side of the ethmoidal labyrinth [1].

A concha bullosa defines the pneumatization of a nasal turbinate. Although the term is commonly used to indicate the pneumatization of the middle turbinate, other nasal turbinates (inferior, superior, and supreme) have also been found to be pneumatized [2-15].

Sagit et al (2008) reported the first case of the pneumatization of six turbinates [16]. Fidan (2012) also assumed that the "pneumatization of all turbinates in a person has not been published yet" and suggested the term "panconcha bullosa" for such pneumatization of all six turbinates [17]. Ozturan et al (2013) failed to document these two previous reports and presented a case with "bilateral triple turbinate pneumatisations," noting, "Despite an extensive search of the medical literature, we could locate only one description of extensive turbinate pneumatisation involving anywhere near as many conchae as in our patient" [18].

Moreover, Bergman's Comprehensive Encyclopedia of Human Anatomic Variation [19] indicated only that the "pneumatization of several turbinates in the same patient has been reported," citing the reports of Sagit et al (2008), Ozturan et al (2013), and a third paper in which Pittore et al (2011) reported only a case of concha bullosa inferior [10], and not a panconcha bullosa. The reports of Fidan (2012) and Toplu et al (2013) were omitted [15, 19].

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We therefore report the fifth known case in the literature of the pneumatization of all nasal turbinates. To our knowledge, it is the first such case being documented in cone beam computed tomography (CBCT) and not on coronal CTs, as well as the first case in which findings were equally documented with bi-dimensional multiplanar reconstructions (MPRs) and three-dimensional volume renderizations (3DVRs). Nevertheless, we hereby report the pneumatization of eight, and not six, turbinates, as well as Santorini's first supreme turbinate was also found to be bullous.

ANATOMIC VARIATION

A retrospective CBCT study of the archived files of a 52 years old female patient was performed.

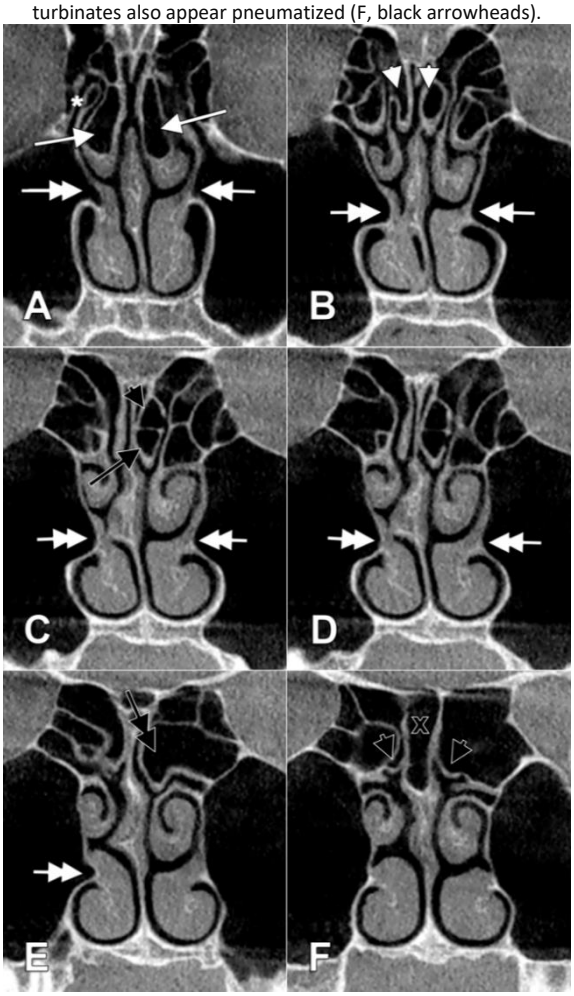
The subject had been scanned using a CBCT machine – iCat (Imaging Sciences International), the DICOM file being further documented with the Planmeca Romexis Viewer 3.5.0.R software, according to a previously detailed protocol of study [20-23]. We evaluated the nasal turbinates in all the three anatomical planes, as well as the three-dimensional volume renderizations. Relevant anatomical features were exported as image files. The patient has given written informed consent for all medical data to be used for research, provided they are anonymized.

In the case hereby reported, a Type 5 Mladina nasal septal deformation was found, with a horizontal septal spur sticking laterally and deeply in the right nasal fossa between the middle and inferior turbinates (Figure 1). The postero-superior part of the nasal septum accommodated an anterior (septal) recess of the right sphenoidal sinus. However, the septum did not appear deviated.

The inferior turbinates were bilaterally pneumatized by conchal recesses of the maxillary sinuses (Figures 1A–E, 2, 3F, 4, 6). The conchal pneumatization penetrated the horizontal segment of the lamellar part of each turbinate (Figure 6), with a 6.10 mm depth on the right side, and 3.02 mm depth on the left side (on coronal slices). These inferior conchæ bullosæ were located between the ethmoidal and maxillary processes of the inferior turbinate (Figure 6).

We also found bilateral pneumatizations of the middle turbinates (Figures 1A, 3, 4). These were both extensively pneumatized (true or extensive concha bullosa media) and were draining in the middle meatus on that side. An uncinate bulla was found unilaterally on the outer side of the right concha bullosa media (Figure 1A). It was pneumatized from an anterior adjacent lacrimal cell located, in turn, posterior to the agger nasi cell.

Figure 1: Anterior to posterior series (A–F) of coronal MPRs identifying the eight turbinates' pneumatizations. The beaked-shape pneumatization of each inferior turbinate is identified (A–E, white double-headed arrows). The bilateral concha bullosa of the middle turbinates (A, white arrows) is of extensive type, lamellar and bulbous. A right uncinate bulla is found (A, *). The superior turbinates are bilaterally pneumatized (B, white arrowheads). A horizontal septum divides the anterior part of the left superior concha bullosa in two chambers, superior (C, black arrowhead) and inferior (C, black arrow). Both drain into the upper nasal meatus (C, D). A third posterior chamber of the left superior concha bullosa (E, black double-headed arrow) appears as a medially evaginated posterior ethmoid air cell. On both sides of an anterior (septal) recess („X") of the right sphenoidal sinus the rudimentary supreme turbinates also appear pneumatized (F, black arrowheads).



We also observed a bilateral concha bullosa superior (Figures 1B–E, 3-6). The conchal air space was narrow in the right side (1.75/4.01 mm on coronal slices). The left concha bullosa superior was larger (4.81/11.35 mm on coronal slices) and septated by an overturned "Y"-shaped septum (Figures 3A, 5).

Thus, it consisted of three chambers: antero-superior, antero-inferior, and posterior. The anterior chambers were draining in the left superior nasal meatus (Figures 1C, D).

Figure 2: Axial MPR demonstrating the bilateral inferior concha bullosa (double-headed arrows).

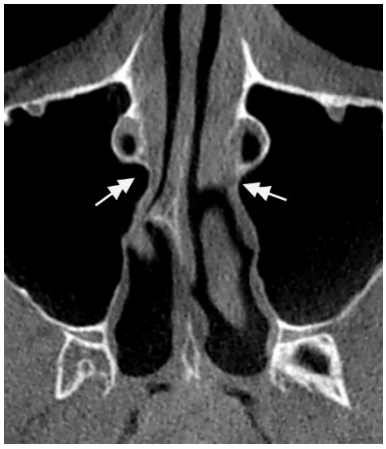
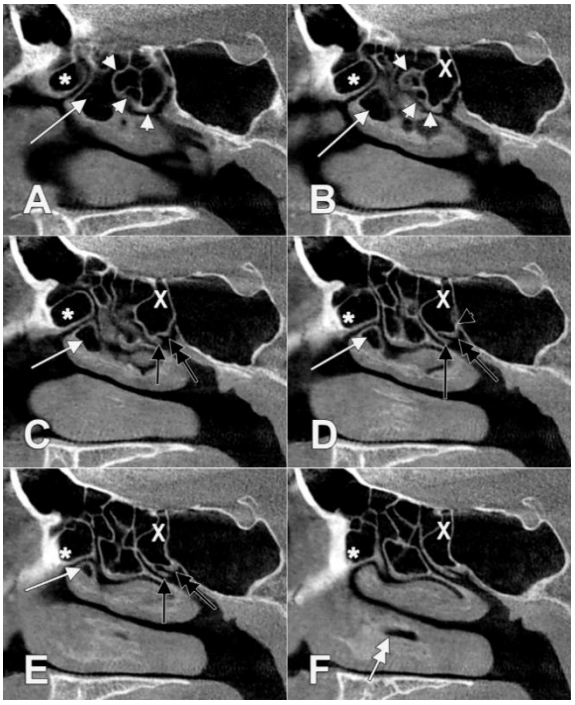


Figure 3: Lateral to medial series (A–F) of sagittal MPRs of the left lateral nasal wall. An agger nasi cell (A–F, *) is found anterior and superior to the anterior end of the middle nasal turbinate. Posterior to it lies the middle concha bullosa (A-–, white arrow). The inferior concha bullosa is indicated (F, white double-headed arrow). The superior concha bullosa is double-septated and consists of three chambers (A, B, white arrowheads): two anterior and one posterior. The posterior one is a conchal recess of a posterior ethmoid air cell (B–F, „X"), as is the supreme concha bullosa (D, black arrowhead). The superior (black arrow) and supreme (black double-headed arrow) turbinates are indicated (C–E).

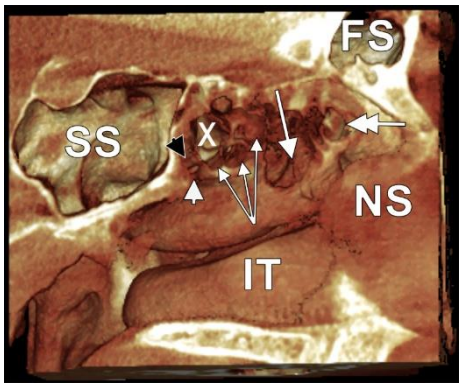


The posterior chamber appeared as the extension (conchal recess) of a posterior ethmoid air cell which, in turn, was also extending to form the left concha bullosa suprema (Figure 3).

As the concha bullosa suprema was also found on the right side (Figures 1, 3–5), we recorded this variation as bilateral. The rudimentary supreme conchæ bullosæ appeared as small diverticula of the respective posterior ethmoid air cells.

Figure 4: Sagittal MPR of the right lateral nasal wall crossing thorough all four conchal pneumatizations: inferior (double-headed arrow), middle (arrow), superior (white arrowhead), and supreme (black arrowhead).

Figure 5: Three-dimensional sagittal volume renderization of the left lateral nasal wall. The superior concha bullosa is separated by septa in three chambers (diverging thin arrows). A posterior ethmoid air cell ("X") pneumatize the posterior chamber of the superior turbinate (white arrowhead) and the supreme turbinate (black arrowhead). The thick arrow indicates the middle concha bullosa and the double-headed arrow—the agger nasi cell. FS: frontal sinus; SS: sphenoidal sinus; IT: inferior turbinate; NS: nasal septum (partly).



Agger nasi cells were found bilaterally. The right one was draining in the frontal recess and the left one in the ethmoidal infundibulum (Figure 7).

DISCUSSION

As was already discussed in the Introduction, reports of panconcha bullosa, such as this one, are rare. The nasal

septum typically deviates away from an asymmetrically enlarged dominant concha bullosa [7].

Nasal septum deformities were classified by Mladina, the left-sided deformities being more prevalent than the right-sided ones [24]. According to these authors, type 5 indicates "an almost horizontal septal spur that sticks laterally and deeply into the nasal cavity," the opposite side of the septum being flat [24].

This corresponds to the septal morphology we found in the present case. The absence of an important septal deviation indicates that we encountered a case of bilateral pneumatizations balanced in size.

Figure 6: Three-dimensional coronal volume renderization of inferior turbinates (ITs). On each side, conchal recesses of the maxillary sinuses (MS) penetrate the turbinate roots (double-headed arrows) to form communicating lamellar inferior conchæ bullosæ. These pneumatizations are located on each side between the ethmoidal process of the ITs (1), which further articulates with the uncinate process (2), and the maxillary process of the ITs (3).

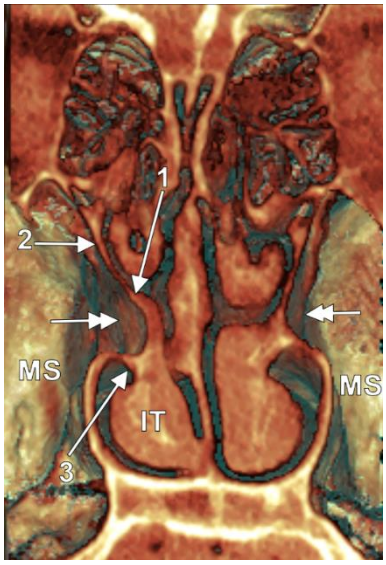


Figure 7: Sagittal MPRs demonstrate bilateral agger nasi cells (*). The right one communicates with the frontal recess (arrow), and the left one drains in the ethmoidal infundibulum (arrowhead).

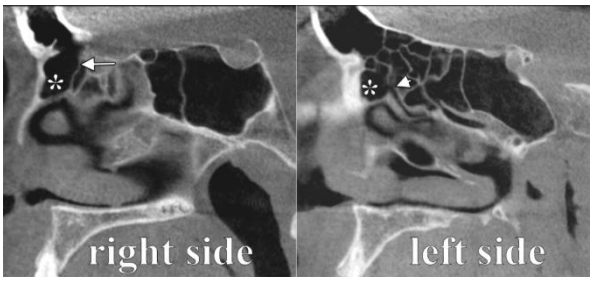
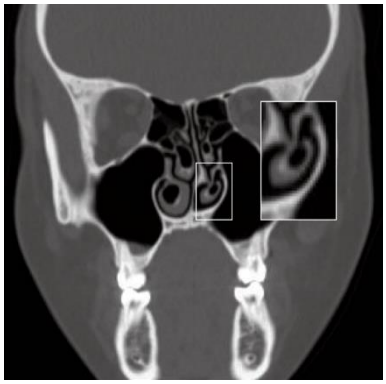


Figure 8: Reprinted from Hindawi – Turhan San, Selma San, Emre Gürkan, and Barış Erdoğan, "Bilateral Triple Concha Bullosa: A Very Rare Anatomical Variation of Intranasal Turbinates," Case Reports in Otolaryngology, vol. 2014, Article ID 851508, 2 pages, 2014. doi:10.1155/2014/851508, which is licensed under CC BY. We indicate (inset, digitally magnified) that the so-diagnosed left inferior conchal pneumatization is merely a strongly curved inferior turbinate, due to a septal spur that pushes the left inferior turbinate.



According to Kiroglu et al (2007), the pneumatization of the upper and middle nasal conchæ is commonly encountered, but a concha bullosa inferior is very rare [25]. Koo et al (2017) documented 1188 sides and found a unilateral concha bullosa media in 17.3%, a bilateral concha bullosa media in 36.4%, a unilateral concha bullosa superior in 11.3%, a bilateral concha bullosa superior in 27.4%, and concha bullosa inferior in only 1.0% [13]. A supreme concha bullosa was reported, to our knowledge, only once, by Măru et al (2015) [2].

The first report of an inferior concha bullosa as an anatomic possibility was that of Zinreich et al (1988), while the first paediatric case with a concha bullosa inferior was assumed by Kiroglu et al (2007).

Bolger et al (1991) evaluated in 202 patients the common anatomical variants of the middle nasal turbinate, such as its pneumatization and the paradoxical curvature [26]. In 53% of patients, the authors detected concha bullosa media [26]. They further classified the respective pneumatizations according to their location, within the lamellar part of the middle turbinate, or in its bulbous part, or in both these. It resulted in a classification of concha bullosa media, lamellar, bulbous, and extensive or true, respectively [26].

Further, Yang et al (2008) extended the classification of Bolger et al (1991) to the inferior turbinate [27]. Yang et al (2008) evaluated 59,238 sinonasal CTs and contributed 16 patients with concha bullosa inferior (pneumatized inferior turbinate) to the 28 cases they had already documented as reported at that time [5, 28-41]. They found that in relation

with the maxillary sinus, a concha bullosa inferior can be either communicating or non-communicating [27]. This equally feeds the hypothesis of misinvaginated epithelium within the double chondral lamella of the developing inferior turbinate (the non-communicating type) and the hypothesis of maxillary sinus extension within the inferior turbinate (the communicating type) [27]. The communicating type of a concha bullosa inferior was found in 8/18 pneumatized inferior turbinates by Yang et al (2008), thus in 44% of the series. Yang further subdivided the communicating type of a concha bullosa inferior into three subtypes, according to the degree of communicative pneumatization (27). These are (a) a subtle type with a beaked shape of pneumatization, (b) a moderate type in which the pneumatization fills the superior part of the vertical plate of the inferior turbinate, and (c) a marked type, in which the pneumatization completely fills the vertical plate of the turbinate [27]. It is interesting to note in this context a study by Măru et al (2013) in which the authors investigated 100 cases documented by CBCT and found unilateral extensions of the maxillary sinus within the inferior turbinates in 6% and bilateral extensions in 16% , which they termed conchal recesses of the maxillary sinus. Although these last authors did not indicate these recesses as pneumatized turbinates, it is bona fide the same morphology previously described by Yang et al (2008) as pneumatized inferior turbinates. According to the Yang classification, in the case we report here, the inferior conchæ bullosæ we found are of the communicating lamellar type, i.e., the moderate subtype. They can be equally classified as conchal recesses of the maxillary sinuses, however. Such communicating conchal pneumatizations were also indicated as common cavities of the maxillary bone and inferior turbinates [42], thus antro-, or sino-conchal cavities.

One should keep in mind that, according to Yang et al (2008), pneumatized inferior turbinates could be either communicating with the maxillary sinus, or non-communicating. Therefore, a concha bullosa inferior that drains in the inferior nasal meatus is a false one. We support this comment with the report of a “bilateral triple concha bullosa” of San et al (2014), who documented such a concha bullosa inferior only on coronal CT in a four-reference paper in which the study of Yang et al (2008) was not quoted. This is a false concha bullosa inferior that communicates with the inferior nasal meatus, and not with the maxillary sinus; narrowing of the turbinate concavity is rather due to a septal spur on that side (Figure 8). The left nasal spur (Figure 8) corresponds to the apex of maximum left septal deviation and is higher than the right concha bullosa inferior. Therefore, the concha bullosa inferior was not bilateral, as is supported by Tomblinson et al (2016), who demonstrated that the apex of maximum nasal septal contralateral deviation is positioned more superiorly when a unilateral concha bullosa is present and that the severity of septal deviation increases in direct proportion to the concha bullosa’s width [7]. Therefore, studies of conchal pneumatization should be evaluated in three dimensions and carefully documented.

In this study, we documented a septated concha bullosa superior that was not, to our knowledge, previously reported. There were, however, a few reports of septated concha bullosa media. The first septated concha bullosa media was found unilaterally and reported by Yanagisawa et al (2008) in a patient elected for functional endoscopic sinus surgery [43]. The second such case was reported by Perić et al (2009), who found a unilateral tri-cameral concha bullosa media [44]. A tri-cameral concha bullosa media was also reported later, by San et al (2013), and was also unilateral [45]. Septation of the middle turbinate is not significantly related to sinonasal pathologies [46]. As supported by our results, the septation of turbinates is not a specific feature of the middle one. Further studies should carefully observe whether the observed conchæ bullosæ contain septa that determine multiple drainage pathways of such pneumatizations.

CONCLUSION

In conclusion, the pneumatization of all nasal turbinates appears as a rare anatomic variation that sums the individual possibilities of the pneumatization of nasal turbinates. Conchæbullosæ are better documented in CBCT and in all three dimensions and, in turn, are of better use for surgeons dealing with functional endoscopic sinus surgery.

Conflict of interests

The authors declare that they have no conflict of interest.

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CLINICAL PRACTICE

Study on the applications of digital smile design planning technique in complete dentures

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Abstract: *One of the most obvious trends in current prosthetic dentistry is the introduction of digital techniques. Of these, the techniques that offer the possibility of aesthetic prefiguration of the final prosthetic result are of particular interest to the dentist.*

The aim of this paper was to verify the applicability of the Digital Smile Design application, which is the most commonly, used aesthetic prefiguration application, an application designed for the dentate patient, to full edentulism. Two groups of full edentulous patients were formed: the first group, complete denture carriers, dissatisfied with the aesthetic aspects, that they wanted to improve in the new denture, and, the second group, newly bimaxillary full edentulous patients with high aesthetic expectations. Thus, on the first group, the tests were performed on the existing denture, and the second one with the maxillary occlusion template. However, the view of the final result offered the patients the chance to have a realistic picture of their desires regarding the shape, color, size of the teeth, which were inadequate in relation to age and facial typology. The use of this application also improves communication with the dental laboratory. In full edentulism, the current DSD technique can be used with some limitations and the use of it in this field needs more complex studies on larger groups of patients.

Keywords: *digital techniques, smile design, complete denture*

INTRODUCTION

Nowadays, dental aesthetics is becoming more and more a subject of interest in dental practice for those patients who want to improve their smile appearance.

If, some time ago, the esthetic requirement was the prerogative of younger people, now, given the increased time of active life, with the increase of life expectancy, this requirement is frequently found among seniors [1], including the full edentulous ones or those in the imminent moment of full edentulism.

Ageing causes a series of major aesthetic disorders, such as altering the soft parts of the face, emphasizing the perioral lines, oral commissures, nasogenian and mentolabial folds, the loss of elasticity of the tissues and the oro-facial muscles [2]. These signs of physiological aging are associated with the pathological signs following the installation of full edentulism. Oral commissures are lowered, and the mouth slit becomes a line with superior convexity. The lower part of the face is diminished, the prominent menton, thus generating a concave profile [3]. To meet patients' requirements, in order to improve the attractiveness of smiles, practitioners must perform a complex facial assessment in an objective and standardized manner. The fundamental criteria for aesthetic analysis include facial, dental, and dento-gingival parameters.

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Nowadays, digital smile simulation programs have been implemented for both research and clinical practice. These are multifunctional tools that strengthen the way of analysis and diagnosis of the facial and dental parameters, improve communication between team members, allow the education and motivation of patients by visualizing the final results of the proposed treatment solutions, and also increase the degree regarding treatment acceptance, the most commonly used technique within current practice being Digital Smile Design DSD [4-6]. Another smile design digital application for dentate but also for the edentulous patient, which takes into consideration patient's personality is Visagismile. Based on visagisme concept (Philippe Halawell), this digital application helps the dentists in their prosthetic work in order to obtain the best esthetic result reflecting patients' personality [7,8]. Designing the smile based only on mechanical parameters such as shape and size of the face will give a monotonous and stereotype appearance whereas customizing it with the person's attitudes and behavior will make it more life-like [9].

However, it addresses fixed dentures retained on teeth or implants [10]. Of course, the implant based dentures, in particular maxillary overdentures retained on implants, a solution with higher esthetic degree by compensating for the horizontal resorption of the vestibular slope through the denture base, supporting the soft parts, remains the first treatment alternative [11] especially for patients who find themselves in the imminent moment of becoming total edentulous. However, this alternative is often not possible due to extensive surgery, poor health, or high costs, the only solution remaining the conventional complete dentures.

When analyzing specialized literature, we observed that no publications tested the applicability of these systems through conventional complete dentures therapy. Thus, the purpose of our study was to check the possibilities and limitations of digital smile simulation applications regarding the total edentulous patient with conventional complete denture.

MATERIAL AND METHOD

The study was conducted between September 2017 and September 2018 on a group of 12 patients, 8 women and 4 men, aged 40 to 80 years, full bimaxillary edentulous, with and without conventional complete dentures, being treated within the Complete Denture Clinic of "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

The inclusion criteria of patients in the study were represented by the increased aesthetic requirements and expectations, but also the desire to preview the result before the treatment itself. The exclusion criteria were represented by patients manifesting disinterest in aesthetics and by uncooperative ones.

All 12 patients who participated in the trial have expressed and signed an informed consent regarding their participation in the study, publication of personal data and photos.

The working protocol consisted in applying and adapting the digital smile planning workflow to the full edentulous patients using the Digital Smile Design application (DSD Free App by Christian Coachman).

The group of patients was divided in 2 categories in order to compare results: patients with existing dentures, on whom simulations were performed, and patients without dentures, being at the clinical stage of determination of intermaxillary occlusion relations, for whom the upper occlusion template was used.

Patients were clinically examined and evaluated for facial features and smile elements, following these aspects:

- Facial features: Shape and Face Size: length/ width, skin, hair, and eye color.

- Smile components: Labial line of smile, smile arc, upper lip curvature – mouth commissures located at the same level (yes/no), buccal corridor – visibility in mm and gingival smile visibility.

Following extraoral clinical examination, each patient received extra and intraoral photos as indicated by the DSD specific working protocol, which we briefly described: uploading photos, calibration of the frontal facial photo, identification and positioning of the incision line and the lines corresponding to the distal faces of the maxillary central incisors, identifying and marking the labial line of the smile, the smile arc and recording the dimensions of the maxillary central incisors, choosing the appropriate teeth template according to the patient's facial typology and preference, generating the smile frame according to the references set in the previous steps and starting the smile simulation, marking the upper lip contour, choosing the teeth color from a series of four pre-set application shades and individualizing it, applying the false gum and individualizing its color, visualization of the final result.

RESULTS AND DISCUSSIONS

DSD simulation on the existing denture and definition of aesthetic elements that corresponded to the patients' aesthetic expectations were performed for the first group.

Pacient I.E., female, 80 years old, disatisfied with the existing complete denture, due to the colour of the artificial teeth

(Figure 1).

Figure 1: Patient disatisfied with the existing complete denture due to color of artificial teeth

Facial features:
- Face shape: oval;
- Face size: 18 cm long, 16 cm wide;
- Skin color: dark;
- Hair color: brown;
- Eye color: brown.

Smile components:

1. Labial line of smile is at the level of the free gingival border and reveals the entire cervico-incisal height of the maxillary frontal teeth and the interdental papilla.

2. Smile arc is a curved line parallel to the upper border of the lower lip.

3. Upper lip curvature - mouth commissures are at the same level as the lower border of the upper lip and thus they do not have a curve, being straight.

4. Buccal corridor - visible on both sides - 3 mm.

5. The patient does not have a gingival smile.

We uploaded the frontal extraoral photos in smile and with apertures, while the patient had the maxillary denture applied to the prosthetic field, but in the smile simulation, we only used the frontal facial photograph. Calibration of the frontal facial photograph that captures the patient's smile with the maxillary denture was applied to the prosthetic field.

Then, the identification and positioning of the incision line and the lines corresponding to the distal faces of the maxillary central incisors was performed.

Afterwards, the identification and marking of the labial line of the smile, the smile arc, and the recording of the dimensions of the maxillary central incisors were performed.

We roughly established the width of the maxillary center incisor at 8 mm and its length at 10 mm and the application setting at an 84% ratio between width and length. These dimensions were going to be subsequently modified in the smile simulation process for a suitable adaptation of the teeth templates that were pre-set by the application to the elements of the facial assembly.

The next steps were the choice of the appropriate teeth template according to the patient's facial typology and preference, the generation of the smile frame according to the references set in the previous steps and the start of the smile simulation.

For patient I.E. we proposed three types of teeth templates: framing the templates in the smile frame, over the patient's pre-existing teeth, and altering their predefined shape, position, size, and color, depending on the patient's needs and preferences, as well as on the facial, dental, and gingival features. Then, we proceeded by marking the upper lip contour and choosing the teeth color from a series of four pre-set application shades and individualizing it. The last step consisted of applying the false gum and individualizing its color.

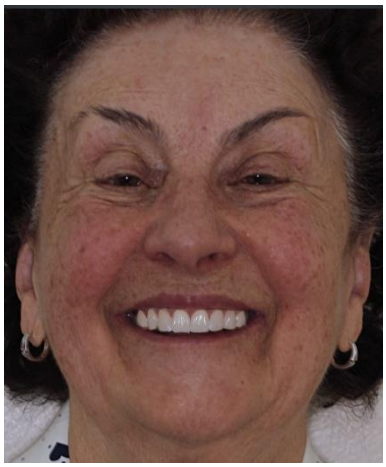
Final result

While smiling, the patient discovered the entire cervico-incisal height of the maxillary frontal teeth, thus allowing the proper application of the DSD working protocol, which required such a smile for a proper simulation of the smile and the achievement of aesthetic results. It was possible to keep the initial size of the teeth without distorting it, in order to allow the teeth to fit into the smile frame.

The teeth templates were selected in agreement with the patient, having a similar shape as the original ones. The patient was aesthetically pleased with the old denture, showing the desire to preserve the same aspect, but we tried to suggest other colors for the teeth, more suitable for the

skin, eyes, and hair. During her first session, the patient had high aesthetic expectations and wanted a new denture with a teeth color that was much lighter than the initial one, very unaesthetic and inapropriate with her age. However, with the application, the patient being able to preview the final result, she understood the shortcomings of a lighter nuance for the teeth and finally opted for a color appropriate to her age and facial typology.

Figure3: Final result patient I.E.



Patient Ș.M., male, 40 years old, bimaxillary full edentulous with great aesthetic expectations (Figure 3).

Figure3: Facial features

Facial features:
- Face shape: oval;
- Face size: 21 cm long, 18 cm wide;
- Skin color: light;
- Hair color: black;
- Eye color: black.

Smile components:

1. Labial line of smile: it is low and reveals less than 75% of the cervico-incisal height of the maxillary frontal teeth.

2. Smile arc is a curved line almost parallel to the upper border of the lower lip.

3. Upper lip curvature - the right mouth commissure is at the same level as the lower border of the upper lip, and the left one is located below, thus the upper lip has a downward curvature on the left side, while the right side is straight.

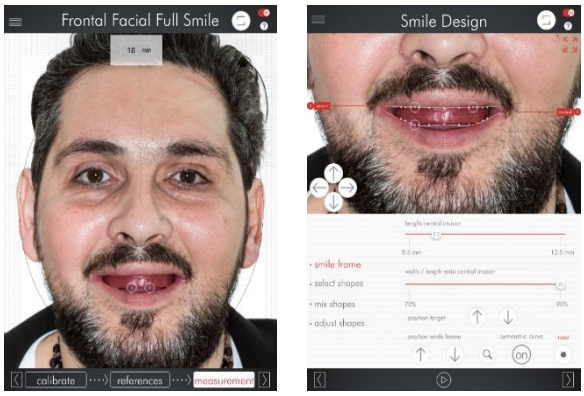
4. Buccal corridor – poorly visible on both sides – 2 mm.

5. The patient does not have a gingival smile.

DSD working protocol with example working on the maxillary template (second group).

Figure4: Frontal facial full smile

Figure5: Frontal facial full smile and smile design



We roughly established the width of the maxillary center incisor at 9 mm and its length at 9.2 mm, the application setting a 90% ratio between width and length. These dimensions were going to be subsequently modified in the

smile simulation process for a suitable adaptation of the teeth templates that were pre-set by the application to the elements of the facial assembly.

The next step consisted of choosing the appropriate teeth template, according to the patient's facial typology and preference, generating the smile frame according to the references set in the previous steps and starting the smile simulation itself (Figures4, 5).

For patient Ș.M., we proposed 3 types of teeth templates: framing the templates in the smile frame, over the patient's pre-existing teeth, and altering their predefined shape, position, size and color, depending on the patient's needs and preferences, as well as on the facial, dental and gingival features. Then we proceeded by marking the upper lip contour and choosing the teeth color from a series of pre-set application shades and individualizing it. The last step consisted of applying the false gum and individualizing its color.

Figure6: Final result patient Ș.M.

Taking a picture with the maxillary template allowed us to simulate a gingival smile because it had a very good hold on the prosthetic field.

The occlusion template gives total freedom in positioning the reerence points such as the incisal line, the lines corresponding to the distal faces of the maxillary central incisors, the labial line of the smile and the smile arc.

In addition, working on the template, we were able to establish several dimensions and shapes of the maxillary frontal teeth, not having to choose a certain size or shape close to the original one, which would cover the artificial teeth on the patient's old denture. In this case, the application guided us to choose rectangular teeth with well-defined angles for the new denture.

Digital Smile Design (DSD App by Christian Coachman) is a tool for digital smile planning used for total edentulous patients, facilitating the choice of shape, color, and size of the artificial teeth, and the possibility to preview the final result before the actual treatment. Moreover, it favors a faster integration of the denture by the patient through his active involvement in the whole process.

In case of full edentulous patients who cannot provide a gingival smile, either because of a poor maintenance of the maxillary denture that comes off the prosthetic field during the attempt to mimic a forced smile, or because of the patient's impossibility to expose the entire cervico-incisal height of the maxillary frontal teeth during the smile, the application has some limitations and requires a considerable reduction in the size of the teeth, in order to allow their optimal fitting within the smile frame, generating less aesthetic results.

Using a photograph with the maxillary template applied to the prosthetic field, instead of the old maxillary denture, within the smile simulation process, will provide the possibility to choose between several types of teeth templates without any limitations regarding the teeth shape close to the one on the old denture, which would generate similar results to the original design of the denture.

Another advantage of using the occlusion template instead of the denture is its good retention. This way, the patient can mimic a gingival smile without the risk of detaching the template from the field, the resulting photograph allowing the correct application of the DSD working protocol and the achievement of superior aesthetic results.

In terms of colors, the application offers a limited range of only four shades with the possibility of changing their features.

In specialized literature, there are still insufficient articles or studies illustrating the expansion regarding the use of digital smile planning applications in total edentulous patients, despite the fact that they can greatly facilitate the design concept of the complete denture, by being able to preview the final result and choose the shape, color, size and arrangement of the artificial teeth according to the facial typology and patient's wishes, thus the patient being involved in establishing the treatment plan.

CONCLUSIONS

Introducing digital techniques to preview the final result of the prosthetic treatment in current dentistry brings important benefits regarding the success of the treatment,

communication with the patient and the dental laboratory.

DSD technique is a tool used in a relatively small percentage in current practice by dental practitioners, only to the dentate patient, which requires the use of digital photographs, templates, and digital applications.

In full edentulism, the current DSD technique can be used with some limitations related to: the dynamics of the lips in smile, execution of the original photographs for which the denture should have a good retention on the prosthetic field and the proposal of a limited number of templates that do not satisfy all facial typologies.

In full edentulism, the vestibular slope of the denture is an important part of its aesthetics, but the application cannot simulate it.

However, the preview of the end result offers the patient the chance to have a realistic picture of his desires, regarding the shape, color, size of teeth, which are inadequate in relation to the patient's age and facial typology.

In the future, studies that are more complex are needed on a larger group of patients, in order to make the application more efficient also in the field of full edentulism.

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All authors have equal contribution as the first author.

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VARIA

Determine the medical causes and diseases which led to early and permanent medical disqualification of military cadets

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Abstract: *Background and Aim: Efficient manpower is the most important of military capital, and many studies have shown the relationship between personal health and organizational performance. The preparation of military cadet volunteers to enter aeronautical training and the provision of optimal services in the form of well-trained pilots requires many material spiritual resources that disabilities, make them waste.*

Methods: This is a descriptive, cross-sectional, retrospective study with target population of Iranian air force cadets with early and permanent medical disqualification from 1986 to 2016.

Results: 80 cases were detected, including 25 kills and 55 early and permanent medical disqualification. The main causes were ear, nose and throat, psychiatry and cardiac, respectively. Common diseases include motion sickness, generalized anxiety disorder, adjustment disorder and epilepsy, respectively. The total number of lost service years was 1412 person years and the average of lost service years was 25.67 person years. Most of the lost service years were related to medical causes included ear, nose and throat, psychiatric and cardiac, and diseases included motion sickness, generalized anxiety disorder and adjustment disorder, respectively. Neurologic causes resulted in highest average of lost service years and high blood pressure was the major diseases, respectively.

Conclusions: Due to the lack of similar studies, the results of this study were compared with the data, which extracted from previous studies in the pilot's population. Because of occupational similarity between pilots and cadets, It was expected that the most common causes in pilots and cadets should be the same, but we found that the results are very different.

Keywords: *disabilities, medical disqualification, health, performance, prevention*

INTRODUCTION

Personal resources are the most important capital of armed forces, and many studies have shown the strong links between personal health and organizational performance. In order to increase productivity, organizations have anticipated numerous measures to evaluate pre-employment health and staff health during service. However, health risks remain the most important reducing factor for personal productivity. Disability is defined as the effect of a disorder on physical, mental, and social activity of a person and the work environment and family status are also effective elements. [1, 2, 3] Cadets are the future capital for professional aviation and the sufficient understanding of the medical causes and diseases leading to their early and permanent medical disqualification (EPMD), plays an important role in professional pilot training. According to statics, effective prevention of cardiovascular risk factors in aviation population has a significant role in improving the professional health index of pilots. The study of pilot's work environment and their lifestyle, active intervention, periodic

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regular examinations and screening test for early detection of diseases are among the basic measures. [9] Preparation of cadets to enter pilot's training courses and delivering desirable services in the form of expert military pilots requires 10 million dollars and extreme spiritual costs, and as a result disqualification, it will be a major loss of money and human budget for armed forces [21]. Thus, control of this dilemma has no way except of effective prevention. [1-5]

These days, the economic, mental and social problems caused by morbidity and mortality of armed forces are critical burdens. Regardless of the huge cost to run and maintain of powerful army, the financial and psychological burden of the victims can be devastating for any society. [20] In 1993, the United States army paid 500 million dollars to newly recruited armed forces. [6-7] In a study between 1980 and 1994 on the causes of disability in US air and sea forces, it has been shown that the disable armed forces has led to an excessive cost in about 1.5 million dollars for US army. [1] The federal aviation association(FAA) has conducted a great comprehensive study in 2014 on the causes of medical disqualification for pilots whose results indicate fifteen causes include pectoris angina, bipolar mood disorder, cardiac valve replacement, coronary artery diseases, diabetes mellitus, loss of consciousness with unknown reason, seizure, cardiac replacement, myocardial infarction, permanent pace maker, disabling personal disorder, psychosis, drug abuse and dependency and lack of neural control, respectively. [15] Cadets are the future maker of aviation and can become expert pilots in near future. Unfortunately, so far, there has not been a similar published study in the world, which makes it necessary to accomplish this research more effective and strategic.

The ultimate goal of this study was to determine the causes and diseases which led to EPMD of Iranian Air Force (IRIAF) cadets from 1986 to 2016. Other objectives include determining the exact numbers of cadet and aviation trainee kills in IRIAF, determining the lost service years (LSY) in IRIAF cadets with EPMD (with counting of seven years of aviation training course and a total service duration of 30 years in IRIAF), determination of the average LSY for IRIAF cadets with EPMD and the provision of scientific and practical solution to prevent the EPMD of IRIAF cadets.

METHODS

This study is a descriptive-cross sectional-retrospective research that its target population was the IRIAF cadets with EPMD from 1986 to 2016. IRIAF cadets with medical disqualification from 1986 to 2016, that their relevant official documents have been recorded in medical sessions and their medical profiles were included, but cadets with disqualification due to physical or mental illness and death in non-occupational accidents, non-medical reasons, personal requests or disciplinary punishments were excluded from study.

The ethical approval of this study was issued with registration No: 1016794 by the Ethics Committee of the Aerospace and diving medical faculty in Aja University of medical sciences. This study was conducted by the personal expense of the corresponding author and so, no governmental or military fund was not be used at all.

After obtaining administrative licenses (Allow access to information) and ethics approval (being ethical and maintaining the principle of patient privacy) in research ethics council of aerospace and diving medical faculty (which is subset of AJA University of medical sciences) and coordination with relevant authorities in IRIAF department of health, medical education and services for obtain permission of unlimited access to medical profiles and sessions, in order to maintain the medical confidentiality, the archivist assigned a secret and random code to each participant that was recorded on top of the electronic data forms. Required information including age, year of entry to and excite from service, main medical cause and disease which led to EPMD were inserted in an electronic form designed according to the study requirements. An individual archivist was used to enter data and in order to military and national information security, the total number of IRIAF cadets was not stated.

All of the IRIAF cadets were male and, in few cases, which there were more than one reason for EPMD, only the main cause was considered. Like other similar studies, all medical causes and diseases were classified based on the 10th version of international classification of diseases (ICD-10) and the causes in domain of nephrology and urology have been recorded as renal causes. However, possible differences in the statistics of similar studies may be related to their classification methods for causes and diseases. It should be noted that in any armed force, while training or real battle, casualties are inevitable and so killed cadets are not considered to be among the lost years of service. The data was collected and analyzed by the researcher using Microsoft office software (version 2016) and compared with the results of similar studies in pilot's population.

RESULTS

During the period from 1986 to 2016, 80 cases of early and permanent discharges were obtained from all the medical sessions and medical profiles, including 25(31.25%) kills and

55(67.75%) medical disqualification. The kills also included 17 cadets and 8 aviation trainees. This data are given in Table1.

Table1: Study Data

Occupation	Early and Permanent Discharge	Kills
Cadets	55	17
Aviation Trainees	0	8

Common causes of EPMD in IRIAF cadets included ear, nose and throat (ENT), psychiatric and cardiac, respectively. Their prevalence is depicted in Figure1. Common diseases resulted in EPMD of IRIAF cadets included motion sickness, adjustment disorder, epilepsy and, respectively and shown in Table2.

Figure 1: Causes lead to the EPMD of IRIAF cadets

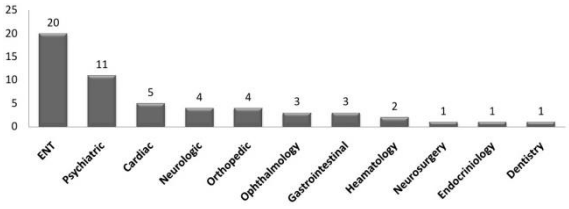

Table 2: Diseases lead to EPMD of IRIAF Cadets

Diseases	Motion Sickness	Generalized Anxiety Disorder	Adjustment Disorder	Epilepsy	High Blood Pressure	Others
N#	18	5	3	3	2	24

As 1412 LSY in IRIAF cadets, the most common causes include ENT, psychiatry and cardiac, respectively. More detail is on Figure2.Common diseases with most of LSY include motion sickness, generalized anxiety disorder, epilepsy, adjustment disorder, and high blood pressure, with detailed statistics in Table 3.

Figure 2: LSY of IRIAF cadets with EPMD according to causes

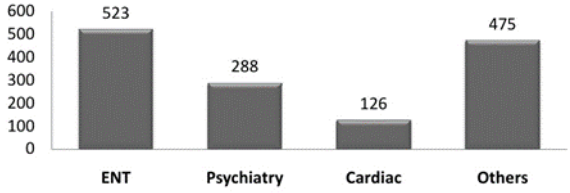

Table3: LSY of IRIAF Cadets with EPMD According to Diseases

Diseases	LSY
Motion Sickness	468
Generalized Anxiety Disorder	137
Epilepsy	82
Adjustment Disorder	74
High Blood Pressure	57
Others	594

The average of LSY in IRIAF cadets with EPMD was 25.67 person years (p/y), the frequency o which based on common causes, including neurology, psychology and ENT with comprehensive information shown in Figure3.

Common diseases resulted in the highest average of LSY in IRIAF cadets with EPMD including high blood pressure, occupational hearing loss, generalized anxiety disorder, epilepsy, and borderline personality disorder, as shown in Table4.

Figure 3: Average LSY of IRIAF cadets with EPMD according to causes

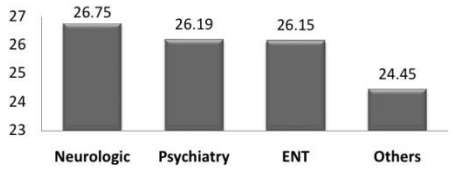

Table4: Average LSY of IRIAF Cadets with EPMD According to Diseases

Diseases	Average of LSY
High Blood Pressure	28.5
Occupational Hearing Loss	27.5
Generalized Anxiety Disorder	27.4
Epilepsy	27.33
Borderline Personality Disorder	26.5
Others	24

DISCUSSION

So far, there has been no investigation into the causes and diseases that lead to EPMD of military or civil cadets in Iran or the world. Therefore, in order to compare the results of this study, the studies in civil and military pilot's population were used, because cadets and pilots have similar occupational and social status. The main reasons for EPMD in IRIAF cadets during our study included ENT, psychiatric, and cardiac, respectively. In the study of Whitton-1984 on United State Air Force (USAF) pilots and navigators, common causes were cardiac and neurologic [16], while in the same

study of Mc Crary-2002, the most common causes in 1995 to 1999, include cardiac, musculoskeletal, neurologic and endocrinology. [11] Also, in the study of Montazeri-2005, the most common causes of EPMD in IRIAF pilots included cardiac and musculoskeletal. [19] In the study of Ghazizade-2010 on the same population in 1992 to 2003, cardiac, nervous-skeletal and gastrointestinal were the most common causes. [18]

In the Dark study with the help of FAA, on medical disqualification of American airlines pilots in 1983 and 1984, the most commonly reported causes were cardiovascular and Neuropsychiatric events. [18] In the Nakanishi study on permanent disability of 260 Japanese crews, significant causes include malignancies, neurodegenerative, cardiovascular, gastrointestinal and musculoskeletal disorders. [8] In the review of trained pilots on Commonwealth countries from 1994 to 2004, the causes of cardiovascular were prevalent. [18] In the Arva study on 257 Norwegian civil pilots, the main causes were cardiovascular, neurologic, musculoskeletal and psychiatric disorders. [10] In the studies of Mitchell-2004 and Evan-2006 on sudden incapacitation of British civil pilots, 36 cases have been diagnosed and half of them were due to cardiac and cerebrovascular disorders and psychiatric disorders have been one of the leading causes. [12, 13] In the Nezami study on 200 civil pilots of Vnukovo airport in Moscow, cardiac, neurologic and digestive have been the main causes of medical disqualification. [9] Common diseases resulted in EPMD of IRIAF cadets, including motion sickness, generalized anxiety disorder, adjustment disorder, epilepsy and high blood pressure. In the Mc crary's review on USAF pilots and navigators in 1995 to 1999, more common diseases were ischemic heart diseases, high blood pressure, back pain, inter vertebral disc disorders and migraine. [11] In the study of Nezami on 200 civil pilots of Vnukovo airport in Moscow, ischemic heart diseases, myocardial infarction, high blood pressure, cerebrovascular sclerotic lesions and peptic ulcer have been common. [9]

The LSY was 1412 (p/y) and the highest was related to ENT, psychiatric and cardiovascular causes, respectively. Diseases with the highest LSY included motion sickness, generalized anxiety disorder, epilepsy, adjustment disorder and high blood pressure. The average of LSY was 25.67 (p/y) and the highest was related to neurologic, psychiatric and ENT causes, respectively. Diseases with the highest average of LSY included high blood pressure, occupational hearing loss, generalized anxiety disorder, epilepsy and borderline personality disorder. In the study of Montazeri-2005, the mean LSY of IRIAF retireds with EPMD was 6.5(p/y). [19] In the Ghazizade study on IRIAF medical disables from 1992 to 2003, the mean LSY was 6.14 (p/y) and the highest was 10 (p/y). [18]

This study, like other studies, has limitations that include: 1) the lack of a comprehensive electronic system for record of medical profile, 2) the possibility of oriented malingering in disorders which have not specific objective diagnostic methods and their detection are subjective, such as motion sickness, migraine, irritable bowel syndrome and etc, and 3) flying device factors(ergonomics and exhaustion), operational and airbase factors(work and rest schedule), Personal factors(life style, physical and psychological capabilities, socioeconomic state, use of legal and illicit drugs and habits), medical factors(medical and psychological advices and hygiene education) are important items which have effective influence on cadets health.

The present study has important benefits such as: 1) at yet, this is the first study on EPMD of military cadets in the world, 2) it has the longest cross-sectional period (35 years) that other longest studies has a period of 10 years, and 3) data presentation in order to kills of cadets and aviation trainees.

At the end, we are suggested that, while it is necessary to conduct similar studies in military and civil cadets and pilots (ever-in non-pilots' flight crew), this advises were recommended to prevent the recurrent medical dispensation of military cadets:

1) Facilitate the comprehensive electronic system for record military cadets' medical profile
2) Upgrading of on-the-job examinations of cadets based on the real requirements
3) Sufficient periodic in-service examination of cadets
4) Lifestyle modifications (habits, nutrition, smoking, use and abuse of drugs and substances, physical exercise etc)
5) Use of medical and psychological counseling (motivation, stress management, high-risk behaviors, risk aversion)
6) Hygiene and health education
7) Regular fitness-based training
8) Updating of flying systems, operational devices and air base facilities (ergonomics, work and rest schedule)
9) Effective and powerful preventive systems with regular updating
10) Improvement of services, which provide with occupational health centers.

List of Abbreviations

Early and Permanent Medical Disqualification (EPMD)
Permanent Medical Exchange in Service categories (PMES)
Iranian Air Force (IRIAF)
Lost Service Years (LSY)
Person Years (p/y)
Federal Aviation Association (FAA)
Myocardial Infarction (MI)

10th International Classification of Diseases (ICD-10)
Generalized Anxiety Disorder (GAD)
Post Traumatic Stress Disorder (PTSD)
Ear, Nose and Throat (ENT)
United State Air Force (USAF)

Declarations

Ethics approval and consent to participate:
The ethical approval of this study was issued with registration N# 1016794 by the Ethics Committee of the Aerospace and diving medical faculty.

Consent for publication:
I (Dr HamzeShahali as Corresponding Author), on behalf of the authors, expresses my satisfaction with the publication of this study in that valuable journal.

Availability of data and materials:
All the data of this study is available and could be accessible for publication by that valuable journal.

Competing interests:
I declare that there is no competing interest about the authors of this study, at all.

Funding:
This research was my special doctorate thesis and so, all the expenses were paid personally. I declare that I did not use any government funds to conduct this study, at all.

Authors' contributions:
Title Presentation, basic researching, approval the research subject, obtaining the necessary permits, collecting data and writing the paper was conducted by Dr HamzeShahali as Corresponding Author and Data analysis, editing and writing part of the article was done by Dr AzadeAmirabadi Farahani as Co-Author, too.

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VARIA

Fever of unknown origin

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Abstract: *Fever of unknown origin (FUO) is a diagnosis that describes a prolonged febrile illness without an established cause, despite investigations. From the first definition of the medical condition (given by Petersdorf and Beeson in 1961) the diagnostic criteria have changed over time, intending to improve the management and outcome of these patients. The main causes of FUO fall into 4 categories: infectious, non-infectious (inflammatory), neoplastic and diverse causes. The etiology of FUO has changed over time, due to ever-changing disease patterns and the development of diagnostic techniques. A significant percentage of patients with FUO remain without an etiologic diagnosis, despite advanced diagnostic tests. Gathering potentially diagnostic clues through history, physical examination and nonspecific key paraclinical abnormalities is the basis for the diagnosis of FUO.*

Keywords: *fever of unknown origin, infections, malignancies, systemic inflammatory diseases*

INTRODUCTION

Fever of unknown origin (FUO) was first defined in 1961 by Petersdorf and Beeson as a medical condition that has the following three characteristics [1]:

• Body temperature 38.3° C (101° F) at three or more successive measurements.

•A duration of at least 3 weeks.

•Uncertain diagnosis after one week of hospitalization.

In the following years, the definition of FUO changed. Immunosuppressed patients were excluded from this category because they have etiological peculiarities and require a different therapeutic approach. Thus, the current definition of FUO includes the following diagnostic criteria:

•a body temperature ≥38.3°C (101°F) at least 2 determinations;

•the duration of illness for ≥ 3 weeks or recurrent febrile episodes for ≥ 3 weeks;

•the absence of immunosuppression, defined as HIV infection or known hypogammaglobulinemia, treatment with prednisone 10 mg or equivalent for a duration ≥ 2 weeks in the previous 3 months before the onset of fever, and neutropenia for a period ≥1 week 3 months before the onset of fever;

•uncertain diagnosis, despite a rigorous case history and clinical examination and the following laboratory investigations: complete blood count, erythrocyte sedimentation rate, ferritin, three blood cultures, tuberculin skin test, C-reactive protein, electrolytes, total proteins,

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protein electrophoresis, creatinine, microscopic urinalysis, urine culture, creatine kinase, rheumatoid factor, antinuclear antibodies, alkaline phosphatase, lactate dehydrogenase, aminotransferases, chest X-ray, abdominal ultrasonography.

EPIDEMIOLOGY

Scientific and technological progress in the microbial diagnosis and classification of the pathogenesis of rheumatic diseases have led to a higher rate of etiological diagnosis among patients with fever. Thus, if in 1930, 75% of patients with fever had no identified causes, nowadays-true FUOs are uncommon [4, 5]. The causes of FUO vary greatly with age. Among children, the most common are viral infections, while among adults' multisystem diseases, such as rheumatic diseases or sarcoidosis, are more likely [5, 6]. Furthermore, geographical region and economic development have an important influence on the etiology of this disease. Thus, in developing countries, the most common causes of FUO are tuberculosis, typhoid fever, amebic liver abscesses and acquired immunodeficiency syndrome (AIDS). In developed countries, the travel of inhabitants to certain areas is a risk factor, resulting in the identification of rare diseases, such as malaria, filariasis, schistosomiasis, Q fever, relapsing fever, Zika virus, fever leash, etc. [5, 7]. Travelers may be infected with microorganisms to which local residents are not vulnerable, because of pre-existing immunity. The diseases contracted while abroad may have a very high incubation period, symptoms (such as fever) occurring after a period from the moment of return. This reduces the chance of an etiological diagnosis [5, 8].

A special category of patients is immunosuppressed patients, such as those with AIDS or known neutropenia. In the case of AIDS, the causes for fever are infections (79%) and malignancies (8%). In 9% of cases, the cause cannot be identified. Regarding the infectious agents, more than half of the cases were due to mycobacteria, especially atypical, the most common being the mycobacterium avium complex (MAC). Regarding malignancies among HIV-infected patients, lymphomas, particularly non-Hodgkin's lymphomas dominate [5]. Febrile episodes associated with neutropenia are most frequently caused by bacteremia, followed by the underlying disease, which has resulted in neutropenia, certain drugs, blood products, and in the case of transplanted patients, allograft rejection [5].

Usually, fever improves with an increase in neutrophils. When fever persists, although the neutrophil count has normalized, the diagnosis of hepato-splenic candidiasis can be considered [9].

ETIOLOGY

The etiology of FUO has changed over time, due to ever-changing disease patterns and the development of diagnostic techniques. The main causes of FUO fall into four categories: infectious, non-infectious (inflammatory), neoplastic and diverse causes [10]. There is also a significant percentage of cases when the etiology of fever remains unknown. A prospective multicenter study estimated the percentage of patients with FUO of 51% [11]. Over time, the frequency of malignancies and infectious diseases as causes of FUO has decreased in favor of noninfectious inflammatory diseases and cases without an etiological diagnosis [12]. From infectious diseases, the most common causes of FUO are tuberculosis and abscesses [13]. The most common malignancies presenting with fever are lymphoma, especially non-Hodgkin, leukemia, renal cell carcinoma, hepatocellular carcinoma, and liver metastasis [13]. Common noninfectious febrile inflammatory conditions correlate with patients' age. Thus, in young patients, systemic juvenile idiopathic arthritis and giant cell arteritis are frequent causes of fever [13]; in older patients, connective tissue diseases or granulomatous diseases are more frequently encountered. Table 1 presents the most common causes of FUO [10, 13, 14].

DIAGNOSIS

FUO remains a diagnostic challenge, considering that there are over 200 disorders that enter in the differential diagnosis. The diagnostic approach therefore should focus on a thorough history of the patient and finding hallmark clinical features that are characteristic of each disease, along with selective non-specific laboratory tests [13]. A detailed history of the patient can provide important diagnostic clues for FUO and should aim to orientate the paraclinical investigations necessary for the positive diagnosis [15]; it should include the history of present illness, data about recent travel, past medical history, drug history, social history and family history (Table 2) [16].

Every FUO category has clinical hallmarks the clinician should search during physical examination [15-18]. Searching potentially diagnostic clues (PDC) during the clinical exam is an important step in the diagnostic workup. PDCs are defined as "all localizing signs, symptoms, and abnormalities potentially pointing toward a diagnosis"[19]:

1. Infectious diseases:
- Spinal tenderness points: suggest spinal tuberculosis, subacute vertebral osteomyelitis;
- Hepatomegaly: Q fever, typhoid/enteric fever, rat bite fever, brucellosis;

- Splenomegaly: miliary tuberculosis, Epstein-Barr virus (EBV) infection, cytomegalovirus (CMV) infection, typhoid/ enteric fever, subacute bacterial endocarditis, Q fever;
- Regional adenopathy: characteristic of cat-scratch disease;
- Epididymo-orchitis/epididymal nodule: EBV infection, renal tuberculosis, brucellosis;
- Cutaneous findings of endocarditis: Osler nodes, Janeway lesions, splinter hemorrhages;
- Heart murmurs: suggest bacterial endocarditis.

Table 1: The most common causes of FUO.

Infections	Malignancies	Systemic inflammatory diseases	Miscellaneous
• Bacterial (tuberculosis, occult abscess, complicated urinary tract infection, osteomyelitis, Q fever, rickettsia infections, enteric fever, brucellosis) • Viral (Epstein-Barr virus, cytomegalovirus, human immuno-deficiency virus) • Parasitic (malaria, toxoplasmosis) • Fungal (histoplasmosis)	• Hepatocellular carcinoma (or liver metastasis) • Leukaemia • Lymphoma, especially non-Hodgkin's • Myelodysplastic syndromes • Multiple myeloma • Renal cell carcinoma • Sarcoma • Colon cancer • Lung cancer • Atrial myxoma	• Giant cell arteritis • Polyarteritis nodosa • Adult Still's disease • Takayasu's arteritis • Granulomatosis with polyangiitis • Mixed cryoglobulinemia • Systemic lupus erythematous • Sarcoidosis • Granulomatous hepatitis • Antiphospholipid syndrome • Behçet's disease	• Drugs (e.g. sulfone-amides, penicillin, nitrofurantoin, vancomycin, antimalarials, H1- and H2-blocking antihistamines, barbiturates, phenytoin, nonsteroidal anti-inflammatory drugs, hydralazine, methyldopa, quinidine, procainamide, anti-thyroid drugs, etc.) • Environmental (metal and polymer fume fevers) • Factitious fever • Disordered heat homeostasis • Alcoholic hepatitis • Venous thrombosis and thromboembolism • Hematoma • Hyperthyroidism and subacute thyroiditis • Pheochromocytoma • Adrenal insufficiency • Familial Mediterranean fever • Tumour necrosis factor receptor-1–associated periodic syndrome (TRAPS) • Hyper-IgD syndrome • Muckle-Wells syndrome • Familial cold autoinflammatory syndrome

2. Malignant diseases:
- Cachexia;
- Diffusely enlarged lymph nodes: lymphoma;
- Erythema nodosum: lymphoma;
- Sternal tenderness points: bone marrow disorders: preleukemia, myeloproliferative disorders;
- Eye examination: Roth spots - lymphoma, atrial myxoma; cytoid bodies - atrial myxoma; retinal hemorrhages - preleukemia.

3. Systemic inflammatory disorders:
- Skin rash: may suggest systemic lupus erythematosus (SLE), adult Still's disease [14];
- Unequal pulse: Takayasu's arteritis;
- Lacrimal gland enlargement: late-onset rheumatoid arthritis (RA), sarcoidosis, SLE;
- Oral ulcers: Behçet's disease, SLE;
- Lymphadenopathy: SLE, sarcoidosis;
- Heart murmur with negative blood cultures suggests a possible diagnosis of Libman-Sacks endocarditis.

4. Miscellaneous diseases. Miscellaneous fevers of unknown origin are often diagnosed based on historical clues rather than clinical findings. Relative bradycardia may suggest drug fever. Cirrhosis is often overlooked as a possible cause of FUO [15, 16]. Splenomegaly may be another important clue that may suggest regional enteritis, cirrhosis or hyper-IgD syndrome.

Paraclinical investigations

Before initiation of any diagnostic tests, treatments, such as antibiotics or glucocorticoids, must be stopped, as they can mask different signs and symptoms and thus delay the diagnostic process.

In any patient with FUO, the following basic laboratory tests are necessary: erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP), hemoglobin, platelet count, leukocyte count and differential, electrolytes, creatinine, total protein, serum protein electrophoresis, alkaline phosphatase, aminotransferases (AST, ALT), LDH, creatine

kinase, antinuclear antibodies, rheumatoid factor, urinalysis, together with blood cultures, urine culture, a tuberculin skin test or Interferon-Gamma Release Assays (IGRA), and imaging studies - chest X-ray, abdominal ultrasonography [19].

Table 2: Key clues to FUO derived from history.

Infectious Diseases	Malignant disorders	Systemic inflammatory diseases	Miscellaneous Disorders
Prior/invasive procedures or surgeries (abscess)	Significant weight loss (2 lbs/week)	Arthralgias	Fever periodicity (cyclic neutropenia)
Dentition (apical abscesses, subacute bacterial endocarditis)	Anorexia	Myalgias	History of lymphadenopathy (Rosai-Dorfman, Kikuchi's disease)
Antecedent/concomitant infections	Post-hot bath pruritus	Dry cough (Giant cell arteritis)	Neck/jaw pain (subacute thyroiditis)
Animal/pet contact (Q fever, brucellosis, toxoplasmosis etc.)	History of adenopathy	Visual disturbances (Giant cell arteritis)	Inflammatory bowel disease (regional enteritis)
Mosquito/tick exposure (malaria, babesiosis)	History of malignancy	Acalculous cholecystitis (SLE, peri-arteritis nodosa)	Alcoholism (cirrhosis)
Rodent exposure (leptospirosis, rat bite fever)	Pel-Ebstein fever-week long high fevers with week-long remissions (Hodgkin disease)	Family history of systemic inflammatory diseases	Medications (drug fever)
Blood transfusions (CMV, HIV)	-	Jaw claudication (Giant cell arteritis)	Prolonged immobility, car trips, flights (thromboembolic disease)
Immuno-suppressive drugs	-	-	-
Nocturia (prostatitis)	-	-	-
High-risk sexual practices	-	-	-
Travel to an endemic area	-	-	-

Complete blood count contains often-overlooked clues, for example, leukopenia, monocytosis, lymphocytosis-relative lymphopenia, eosinophilia, basophilia, atypical/abnormal lymphocytes, thrombocytosis, and thrombocytopenia. In FUO, an isolated alkaline phosphatase elevation may be a clue for lymphoma [20, 21, 22]. Eosinophilia may suggest polyarteritis nodosa, drug fever, or visceral leishmaniasis. Low levels of hemoglobin or hematocrit may point toward occult hemorrhage or hematoma (often retroperitoneal).

After all potentially diagnostic clues retrieved from the patient's history, clinical examination and paraclinical investigations have been identified, the clinician should make a list of the most probable diagnoses; further specific investigations should be guided by these diagnoses.

If the diagnosis remains elusive, more advanced paraclinical investigations may be necessary. In patients with hepatomegaly or splenomegaly, abdominal computed tomography (CT) scan may be useful in detecting abnormalities such as adenopathy, abscesses or masses [23]. Echocardiography is important in case of suspicion of endocarditis or atrial myxoma. Positron emission tomography-computed tomography (PET-CT) scans help detect obscure infectious/neoplastic causes of FUO, for example, lymphomas, Q fever endocarditis or aortic graft infection [24]. Magnetic resonance imaging (MRI) may be useful in case of osteomyelitis. Digestive endoscopy may become necessary in case of suspicion of inflammatory bowel disease or sarcoidosis [25].

Invasive tests, such as lymph node biopsy, bone marrow biopsy, epididymal nodule biopsy, liver or ileal biopsy may have a high diagnostic yield [26-29]. However, these tests should be ordered based on narrowed diagnostic possibilities [30, 31].

TREATMENT

Treatment should be directed toward the underlying cause once a diagnosis is made. Because they may mask, delay or obscure the clinical manifestations, empirical therapeutic trials with antibiotics [32, 33], glucocorticoids, or antituberculous agents should be avoided in FUO, except in immunocompromised, neutropenic patients, in case of severe systemic illness or those with a rapid deterioration (patients with central nervous system or miliary tuberculosis or giant cell arteritis) [34, 35].

CONCLUSION

FUO remains one of the most difficult diagnostic challenges, as the differential diagnosis consists of over 200 malignant/ neoplastic, infectious, rheumatic/inflammatory, and miscellaneous disorders. It is essential for the clinician to

obtain potentially diagnostic clues through a detailed history of the patient, complete physical examination and guided paraclinical investigations. Interdisciplinary consultations may be appropriate in a majority of patients with FUO.

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VARIA

Developing a conceptual model for healthcare services branding in the military hospital

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Abstract: *Introduction: Given fast grow and competition in the healthcare industry, managers and health activists in this sector need to adopt strategies for branding. The purpose of the study was to develop a conceptual model for branding in the healthcare industry to build brand advocacy and positive brand image.*

Methods: The study includes patients referred to a military hospital in May 2018. Data gathered by questionnaire, and 162 surveys were collected by simple random sampling method. To test the hypotheses, the conceptual model was analysed by structural equation modeling with SmartPLS® software.

Results: The affective commitment and brand trust could predict brand advocacy. CSR significantly impacts brand image and affective commitment, furthermore brand attitude and COB significantly impact brand trust. There was no significant relationship between brand trust and affective commitment with brand image.

Conclusion Organizations can improve brand image and establish brand support through investing resources on branding. This study contributes insights for marketing managers in developing successful branding strategies for health care organizations.

Keywords: *branding, health care industry, hospitals, SEM*

INTRODUCTION

Healthcare services are dynamic, competitive and full of turbulence. The number of hospitals and healthcare providers rising continuously and with this situation, the atmosphere becomes more and more competitive. Furthermore, in Iran, hospitals can't advertise themselves (It's a law) for gaining more patients and customers (Maleki, 2016). In the other view, measuring the efficiency of healthcare services is depending on lots of quantitative and qualitative criteria (like capable employees, behavioural and technical interactions, treatment matter, type of patient, public and private services) and because of that, it is hard to measure (Thantry et al., 2006).

Due to the hard situation for hardware and material distinction, organizations prefer to be distinct in software (Kotler & Keller, 2012). Huge different exists between services and product usage and because of that, considering people who use them, is different and need other conceptualization (Wang et al., 2009; Van Riel et al., 2001). Moreover, a brand in the service sector corresponds with "organization" whereas in the production sector correspond with "product" and according to this, conceptualizing in the service sector is essential (Wang et al., 2009; Chahal & Bala, 2012). When a high value of satisfaction and emotional commitment is issue, branding in hospitals that refer to customer needs is crucial (Kemp et al., 2014).

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Health care is one of the most critical services that a patient receives (Kemp et al., 2014). Like brand in product sector that has socio-psychological attributes, for service sector, brand is same and can be inspiring. This means there is a bond between brand and customers (Kemp et al.). The relationship between brand and customer is binary. Developing relationships between brand and customer can be a challenging procedure and in a different pattern. For example, it is possible that nexus among brand and customer is according to simple or emotional cognition (Park et al., 2009; Thomson et al., 2005; Brakus et al., 2009; Grisaffe & Nguyen, 2011). When there is a strong relationship between brand and customer, the organization has a significant advantage in competitive atmosphere and also can immune organization from rivals (Kemp et al.).

The progress of healthcare services and economic development are closely linked; reaching one without other is impossible (Khodadad & Behboudi, 2017). Branding in healthcare services is a newfound phenomenon, but managers are gaining intuition from its competitive advantage. Meanwhile, hospitals as an expensive part of the healthcare industry that assign a high portion of GDP, need more attention and considering (Nasiripour et al., 2010). Branding plays a vital role in service organizations through increasing customer trust, make a better condition to illustrate services, acts as a mean of competitive advantage and gives value to the customers (Chahal & Bala, 2012). Branding in healthcare services requires a strong and organized commitment to presenting unique services. For having successful branding, managers should have a strategy for maintaining and guiding values for making trust and also managing emotion (Kemp et al., 2014; Mangini, 2002).

The purpose of the study was to develop a conceptual model for branding in the healthcare industry to build brand advocacy and positive brand image.

METHODS

This descriptive study surveyed patients who went to a military hospital in 2018 may, using simple random sampling. To determine the minimum sample size before gathering data Kock and Hadaya (2018) suggest the use of the inverse square root method, which estimates 160 samples. After distributing the questionnaires, 162 samples were gathered. The questionnaire that distributed had two parts, first demographic questions and then 28 questions that evaluate survey variables in Likert-5 scale. The research constructs were developed based on prevalidated measures (Table 1). Instrument items were modified by experts in any aspects and rephrased.

Table 1: Validity and reliability

Construct	Cronbach Alpha (α)	Composite Reliability (CR)	Average Variance Extracted (AVE)	Source
Corporate Social Responsibility	0.856	0.920	0.700	Kemp et al, (7)
Brand Attitude	0.849	0.906	0.710	Kemp et al, (7)
Customer-oriented Behavior	0.9	0.929	0.768	Kemp et al, (7)
Affective Commitment	0.883	0.936	0.831	Kemp et al, (7)
Brand Trust	0.890	0.932	0.821	Kemp et al, (7)
Brand Image	0.809	0.872	0.583	Wu (67)
Brand Advocacy	0.739	0.869	0.623	Kemp et al, (7)

After data gathering, single variable and two variables measurement analyses with SPSS® version 25 software and analysis in structural equation modeling calculated with Smart-PLS® version 3.2.7 (Ringle et al., 2015). Cronbach alpha (recommended threshold ≥ .7) (Cronbach, 1951) and composite reliability (CR) (recommended threshold ≥ .7) (Hair et al., 2016) estimated for reliability and average variances extracted (AVE) (recommended threshold ≥ .5) (Fornell&Larcker, 1981) estimated for validity with Smart-PLS®. Results of Validity and Reliability analysis (Table 1) exemplified acceptable value as exceeded recommended threshold, which is an indication of a validated and reliable instrument.

RESULTS

Researchers can figure out data essence with descriptive results. Results of descriptive analysis like gender, age,

education, insurance and hospital familiarity (or use duration) are in Table 2.

Table 2: Demographic statistics

Measure		Frequency	Percentage
Gender	Male	94	53.4
	Female	82	46.6
Age	Under 20	8	4.5
	21-30	54	30.7
	31-40	47	26.7
	41-50	33	18.8
	Above 50	34	19.3
Education	Under diploma	40	22.7
	Diploma	50	28.4
	Bachelor	56	31.8
	Master	24	13.6
	Doctoral	6	3.4
Having Insurance	Yes	170	96.6
	No	6	3.4
Time of being hospital customer or (familiarity)	Under 1 year	78	44.3
	1 year	30	17
	2 years	21	11.9
	3 years	10	5.7
	Above 3 years	37	21

Most participants were male (53.4%), 30.7% were 21-30 years old, 31.8% had bachelor degree, almost every participant has insurance (96.6%), and 44.3% of participants were being hospital customer under one year. Constructs averages and standard deviations are in Table 3.

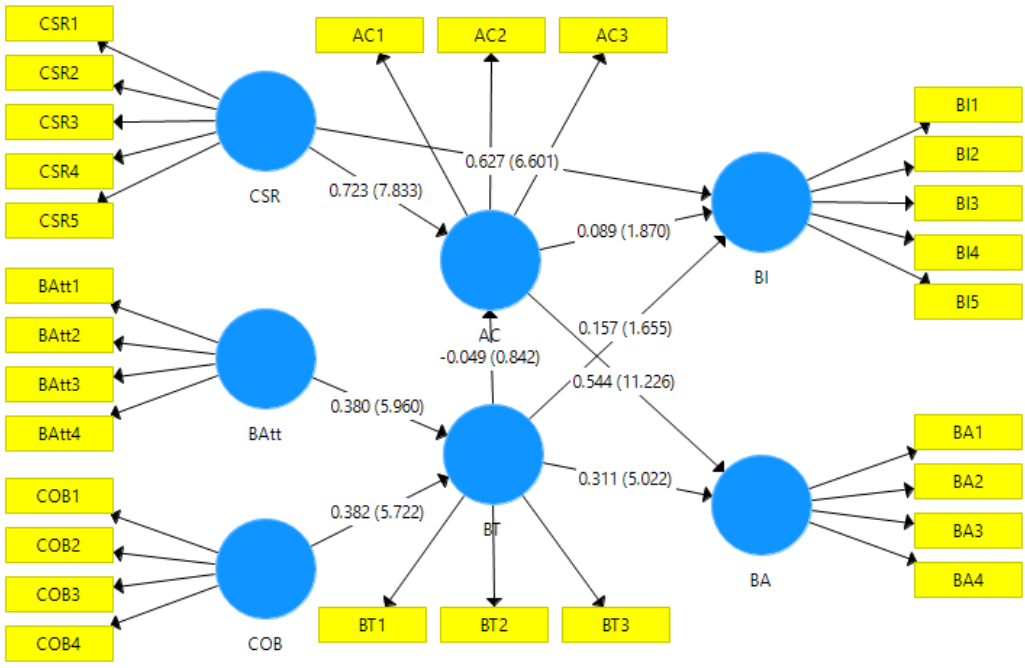
Table 3: Average and Standard Deviation of research constructs

Construct	Average	Standard Deviation
Corporate Social Responsibility (CSR)	3.806	0.971
Brand Attitude (BA)	4.117	0.514
Customer-oriented Behavior (COB)	3.458	1.239
Affective Commitment (AF)	3.448	1.266
Brand Trust (BT)	4.152	1.06
Brand Image (BI)	4.190	0.793
Brand Advocacy (BA)	4.024	0.743

In two variable analyses, researchers used Spearman coefficient correlation. Results of this analysis are in Table 4.

Results of assessing hypotheses using structural model in smart-PLS® (Ringle et al., 2015) (standard and T-values, values in parenthesis in the model) are in Figure 1 which shows H1a, H1b, H2a, H2b, H3 and H4 confirmed and H2c, H5 and H6 rejected. Results are in Table 5.

Figure 1



Structural model assessment: For assessing quality of structural model, R2 considered as criteria for assessing model (Hair et al., 2016) and results show that all variables are more than average (0.33 is the mid number). Table 6 shows the values.

Table 4: Spearman Correlation coefficient, all the numbers are significant at 0.01 level

Construct	CSR	BA	COB	AF	BT	BI	BA
Corporate Social Responsibility	1						
Brand Attitude	0.602	1					
Customer-oriented Behavior	0.501	0.262	1				
Affective Commitment	0.598	0.436	0.334	1			
Brand Trust	0.775	0.467	0.500	0.485	1		
Brand Image	0.712	0.477	0.463	0.481	0.637	1	
Brand Advocacy	0.666	0.445	0.379	0.701	0.627	0.571	1

Table 5

Hypothesis	Independent variable	Dependent variable	Path coefficient (β)	T-value	P-value	result
H1a	Corporate Social Responsibility	Brand Image	0.627	6.601	<0.01	Confirmed*
H1b	Corporate Social Responsibility	Affective commitment	0.732	7.833	<0.01	Confirmed*
H2a	Brand Attitude	Brand Trust	0.380	5.960	<0.01	Confirmed*
H2b	Customer-oriented behavior	Brand Trust	0.382	5.722	<0.01	Confirmed*
H2c	Brand Trust	Affective Commitment	-0.049	0.842	0.420	Rejected
H3	Affective Commitment	Brand Advocacy	0.544	11.226	<0.01	Confirmed*
H4	Brand Trust	Brand Advocacy	0.311	5.022	<0.01	Confirmed*
H5	Affective Commitment	Brand Image	0.089	1.870	0.059	Rejected
H6	Brand Trust	Brand Image	0.157	1.655	0.109	Rejected

* Significant at 99%

Table 6: Model assessment results

Construct	Average
Affective Commitment	0.446
Brand Trust	0.346
Brand Image	0.657
Brand Advocacy	0.553

Finally, according to Tenenhaus, the goodness of fit measured and according to formula

$$\mathrm{GOF} = \sqrt{\mathrm{Communality} \times \mathrm{R}^2} = 0.492$$

calculated that exhibits well goodness of fit (Tenenhaus et al., 2004).

DISCUSSIONS AND CONCLUSIONS

This research aimed to find a pattern for branding in the healthcare industry. Hence, variables like corporate social responsibility, brand attitude, customer-oriented behavior, affective commitment, brand trust, brand image, and brand advocacy recognized and the relationship between them through a conceptual model is investigated. Results showed corporate social responsibility has a significant effect on emotional commitment and brand image; these results are in accordance with recent researches (Kemp et al., 2014; Alrubaiee et al., 2017; Kolade&Ogunnaike, 2014). Brand image mostly previses corporate social responsibility. Therefore, by developing corporate social responsibility, brand image become better in customer mind, and marketing performance will improve. Besides, managers can determine policies and strategies in marketing more efficient due to this results and structures. Furthermore, it is crucial for hospitals to invest and help society, which can create an emotional bond and affective commitment.

According to the results, brand attitude and customer-oriented behavior predicted brand trust, and this is following Kemp et al. research (Kemp et al., 2014). Thus, creating a positive brand attitude and customer-oriented behavior from employees will improve brand trust. Educating employees for having better conduct and behavior with customers can improve customer-oriented behavior. Since the healthcare industry is a personal service and a close relationship exist between parties, trust is crucial due to personal information, mental and physical status exposure to healthcare providers (Kemp et al.).

Brand trust could not predict affective commitment that is in the opposite of previous surveys (Kemp et al., 2014; Patawayati et al., 2013; Feiz et al., 2016). However, it could predict brand support that is in accordance with previous researches (Kemp et al.; Moreira & Silva, 2015; Ansari &Mohammadi, 2016). Results showed with brand trust rise up; brand support will rise simultaneously. Hospitals should focus on high trust level in marketing (Kim et al., 2004). When a customer trust in healthcare service provider, it is possible that he/she continues to use that service provider and suggests it to others. Hospitals should use their sources to invest in developing more trust for creating more brand advocacy. Results showed affective commitment could predict brand advocacy and it is in accordance with previous surveys (Kemp et al.; Moreira & Silva; Feiz et al., Ansari &Mohammadi), so by developing an emotional relationship with customer, he/she becomes a brand advocator and finally, competitive advantage will be created.

This research demonstrates the advantages of branding and establishing a meaningful relationship with customers. Results of this research can help marketing managers and strategic planners. In the healthcare industry, vicinity with a hospital is a key factor for productivity (Beckham, 2000). Nevertheless, with increasing healthcare costs and more options for customers, marketers can act more strategic through creating competitive advantage, and distinguishing customers' needs (Brakus et al., 2009).

Healthcare organizations should effectively put their organization and its brand as a valuable factor in providing unique services. As proposed in this research, corporate social responsibility, affective commitment, customer-oriented behavior, customer engagement (like customer relationship management) and trust, are significant elements to reach this goal.

Although this study can help in creating and developing brands in healthcare services, future research and surveys in this field are imperative. Several limitations have existed in this study. First, as data gathered in a cross-sectional way, no cause and effect relationship can be established about research variables. Investing in a relationship between latent variables through longitudinal data is suggested. Future studies can investigate how the correlation between performance and actual satisfaction can create a connection between brands and consumers. Second, some discordance observed between the results of this research and literature, so the research results should expand cautiously. Third, respondents to questionnaires are from the same region so, gathering data from diverse regions is suggested. Forth, in this study, healthcare providers were limited to hospitals, more scopes like pharmacy, clinics, medical imaging, laboratories, and dental services should be investigated. Finally, respondents may somehow were not in their best physical and mental condition, so this maybe affects the data gathering and analysis.

At least once in a lifetime, people use healthcare services, and because of that, good experience and relationship with providers are crucial. As changing perspective in healthcare services in future years, research opportunity in strategical branding and marketing fields will be plentiful for healthcare service providers.

Conclusion Organizations can improve brand image and establish brand support through investing resources on branding. This study contributes insights for marketing managers in developing successful branding strategies for health care organizations.

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VARIA

Redo procedure following Zenker`s diverticulum treatment – a clinical study

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Abstract: *Introduction: The prognosis of patients with Zenker`s diverticulum is dependent mainly on the type of procedure performed, either surgical or rigid/flexible endoscopy.*

Purpose - evaluation of the redo procedure by reviewing correlations in patients with iterative procedures.

Material and Methods: We included in the study 36 patients with cervicomediastinal Zenker diverticula treated from 2010 to 2017 at two university hospitals: Clinic of General and Esophageal Surgery. Mary Clinical Hospital Bucharest and Department of Diagnostic and Interventional Digestive Endoscopy of the Regional Institute of Hepatology and Gastroenterology Prof. Dr. Octavian Fodor, Cluj-Napoca. For the patients included in the study we studied correlations of patients with symptoms persistence and to those with reintervention with epidemiological, clinical and the treatment criteria.

Results: Postprocedural symptoms we observed in 5 patients of the 29 treated endoscopically and 6 of the seven treated surgically. Statistical correlations were noted in patients with persistent symptoms after surgical treatment ($p < 0.01$) with patients with diverticulectomy (54.5%) ($p < 0.01$), grade III diverticula (36.4%) ($p < 0.01$) statistical association was noticed in patients who have required reoperation and those with persistent symptoms (63.6%) ($p < 0.01$) and with remaining dysphagia (100%) ($p < 0.01$) and those with grade 2 dysphagia persistence (71.4%) ($p < 0.01$), grade 3 diverticula (42.9%) ($p < 0.03$) and the high number of comorbidities. ($p < 0.04$)

Conclusion: Undoubtedly, endoscopic treatment is being used more and more even for those cases that require further treatment. Reoperation has been necessary for patients with persistent dysphagia with voluminous diverticula and a high number of comorbidities.

Keywords: *diverticulum Zenker, endoscopic diverticulostomy, diverticulectomy, iterative procedure*

INTRODUCTION

Zenker`s diverticulum is a cause of morbidity in elderly patients. According to van Overbeek's theory, the predominant role in diverticulum formation has an anatomical predisposition represented by Killian dehiscence weakness. [1] The externally surgical approach has long been the conventional treatment with satisfactory clinical success rates ranging from 80-100%. [2]

The prognosis for these patients depends on the surgical procedure performed. Some authors stated that diverticulectomy patients have a long-term symptom recurrence rate of 2% to 33%. [3] Studies have been published since 1981, which noted that surgical resection or suspension of the pouch performed without a concomitant

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myotomy fails to improve dysphagia and fail to prevent complications or recurrence of the diverticulum, a fundamental consideration of the endoscopic techniques developed later. [4]

Some authors found that patients with endoscopic stapler technique, have a long-term symptom recurrence rate ranging from 0 to 47%. Most authors consider that the reasons for symptomatology after the endosurgical approach is the incompatibility of the dimensions of the septum with the length of the stapler (3 cm). A small diverticulum limits access to the tip of the stapler and it may not be possible to perform a proper myotomy. [5]

Data from the literature indicate a recurrence rate for laser endoscopic diverticulostomy of about 5-25%. [6, 7]

Flexible endoscopic therapy is a relatively new approach compared to rigid endoscopic and surgical techniques. Data from the literature on flexible endoscopic therapy show that symptomatic improvement was observed at 85%, with a symptom recurrence rate of 18-20%. [8-11]

The higher recurrence rate after flexible endoscopic treatment could be caused by incomplete septal dissection. Some authors have shown that the success of the treatment correlates with the length of the septotomy. The risk of perforation by over dividing muscular fibers and difficult identification of the last fibers of the cricopharyngeal muscle could make the endoscopist to leave a residual part of the septum to avoid perforation into the mediastinum. [12]

MATERIAL AND METHODS

We included in the study group 36 patients with Zenker diverticula treated during 2010/2017 in two university hospitals: Clinic of General and Esophageal Surgery Sfanta Maria Bucuresti and Department of Diagnostic and Interventional Digestive Endoscopy of the Regional Institute of Hepatology and Gastroenterology Prof. Dr. Octavian Fodor, Cluj-Napoca.

Inclusion criteria: patients with Zenker diverticulum admitted and treated endoscopically or by open surgery in the hospital during 2010/2017, over 18 years of age, investigated with barium swallow and upper GI endoscopy, patients with a follow-up period of at least 12 months post-procedure.

Exclusion criteria: immunocompromised patients, patients with associated malignant disease, patients lost from post-procedure follow-up.

All patients in the group were investigated by clinical examination, imaging with a barium swallow, upper endoscopy and functional tests with esophageal manometry when this exploration was possible.

Dysphagia was a constant symptom at presentation to the hospital, as well as the main symptom followed in the evaluation of the treatment efficiency. For the assessment of this symptom we used Mellow-Pinkas classification of dysphagia applied to the type of food that cannot be swallowed - grade 0 - No dysphagia, grade 1 - Dysphagia for solids, grade 2 - Dysphagia for semisolid, grade 3 - Dysphagia for solids and liquids grade 4 - Total dysphagia (inability to swallow saliva).

The sizes of the diverticula were appreciated both in centimeters and in degrees according to the Overbeeck classification by corroborating data obtained from the endoscopic exploration and the appearance of the barium contrast imaging. 1 – the small sac that has less than 1 vertebral body in length; 2 –intermediate sac 1-3 vertebral bodies in length; 3 – large sac greater than 3 vertebral bodies in length.

For all the patients surgically treated by cervicotomy, the resection of the diverticulum was performed with myotomy and manual esophageal suture, either single or double layer, or mechanically using a linear stapler. For the patients treated endoscopically, myotomy was performed with creating a wide communication of the diverticulum with the esophagus without resection of the diverticular pouch. A soft, flexible overtube (Cook Medical, Indiana, USA) was used to expose the septum, which was placed over the endoscope, with the short blade placed in the diverticulum and the long blade in the esophagus. After the septum was exposed, it was divided on the midline, from the esophageal lumen to the diverticular pouch, on an average length of 1.5 cm using a Dual knife (Olympus Co., Japan) followed by the closure of the mucosa with clips (2 -5 clips). All patients were clinically and imaged monitored at 3-month intervals during the first post-procedural year.

Study design

For the patients included in the study we studied correlations of patients with symptoms persistence, and to those with reintervention with epidemiological, clinical and the treatment criteria.

Statistical analysis

In comparison with the quantitative data, by the normality of the data, Student's t-test (for two groups with normally distributed data) and Mann-Whitney and Kruskal-Wallis (for data without normal distribution) were used. For the categorical data (synthesized as frequencies and percentages), Fisher exact tests (binary data) and Pearson

Chi-square were used as well as Likelihood Ratio (if 20% of expected frequencies were less than 5). The probability of error of less than 5% ($p < 0.05$) was considered as a significance threshold. The paper also included graphic representations, such as stratified bar type for data synthesized as frequencies and percentages.

RESULTS

Patients included were 29 patients treated endoscopically by myotomy in the Department of Diagnostic and Interventional Digestive Endoscopy of the Regional Institute of Hepatology and Gastroenterology Prof. Dr. Octavian Fodor, Cluj-Napoca and 7 patients surgically treated by resection of the diverticulum associated with myotomy. In the Clinic of General and Esophageal Surgery of St. Mary's Hospital Bucharest. The intraprocedural complications were laminar hemorrhage in three patients during the endoscopic procedure, and one surgically treated patient developed a postoperative hematoma that required drainage. Post-procedural mortality was 0%, and morbidity was 13.88% - five patients (3 patients after endoscopic treatment and 2 patients after surgical treatment).

Persistent postinterventional symptomatology was reported by 11 patients and consisted of the persistence of dysphagia of a lower degree associated or not with the persistence of other symptoms: sialorrhea, regurgitation, nausea, and cough.

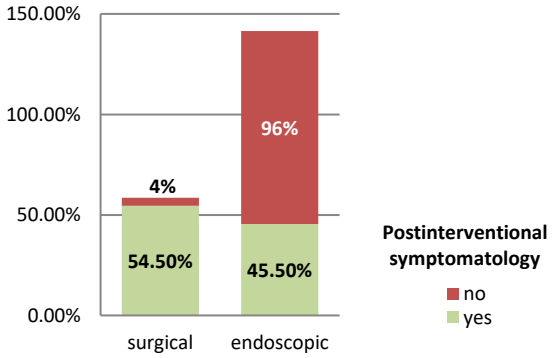
We did not observe associations regarding the correlation of the persistence of post-procedural symptomatology with the patients' sex, initial symptomatology or with diverticulitis. (Table 1)

Table 1: Correlations of post-intervention symptomatology - statistical processing of data

	Post opSymptoms = Yes (N=11)	**Post opSymptoms= No (N=25)**	**P_value (test)**
Sex=M	7/11 (63.6%)	14/25 (56.0%)	0.728773 (Fisher's Exact Test)
Grade			
1 2 3 4	0/11(0.0%) 5/11 (45.5%) 2/11 (18.2%) 4/11 (36.4%)	2/25 (8.0%) 15/25 (60.0%) 7/25 (28.0%) 1/25 (4.0%)	**0.063384** (Likelihood Ratio)
No Symptoms			
1 2 3 4 5	3/11 (27.3%) 1/11 (9.1%) 4/11 (36.4%) 1/11 (9.1%) 2/11 (18.2%)	8/25 (32.0%) 4/25 (16.0%) 11/25 (44.0%) 2/25 (8.0%) 0/25 (0.0%)	0.266965 (Likelihood Ratio)
Proceduresurgicalendoscopic			
	6/11 (54.5%) **5/11 (45.5%)**	**1/25 (4.0%)** **24/25 (96.0%)**	**0.001423** (Fisher's Exact Test)
Pouch resection			
Yes	**6/11 (54.5%)**	**1/25 (4.0%)**	**0.001423** (Fisher's Exact Test)
Div Dimensions			
1 2 3	2/11 (18.2%) 5/11 (45.5%) **4/11 (36.4%)**	12/25 (48.0%) 13/25 (52.3%) **0/25 (0.0%)**	**0.003085** (Likelihood Ratio)
Dysphagia post op			
Yes	**10/11 (90.9%)**	**1/25 (4.0%)**	**0.000000** (Fisher's Exact Test)
Dysphagia Grade			
0 1 2	**1/11 (9.1%)** **4/11 (36.4%)** 6/11 (54.5%)	24/25 (96.0%) 1/25 (4.0%) 0/25 (0.0%)	**0.000000** (Likelihood Ratio)
Hospitalisation			
	7.55±5.5563 **8.00 [2.00, 11.00]**	2.56±1.5832 **2.00 [2.00, 3.00]**	**0.005955** (Mann-Whitney Test)

Post-procedural symptomatology we observed only in five patients out of the 29 endoscopically treated and in six out of the seven surgically treated. The statistical association was observed for the type of intervention practiced – endoscopic or surgical treatment (p <0.01) and the persistence of postintervention symptomatology (endoscopic treatment = 45.5%) compared to those without postinterventional symptomatology (endoscopic treatment = 96.0%) – 96% of patients who did not report persistent symptoms had endoscopic treatment. (Table 1) (Figure 1)

Figure 1: Distribution of patients according to the presence of the post-intervention symptomatology to the surgical or endoscopic procedure type



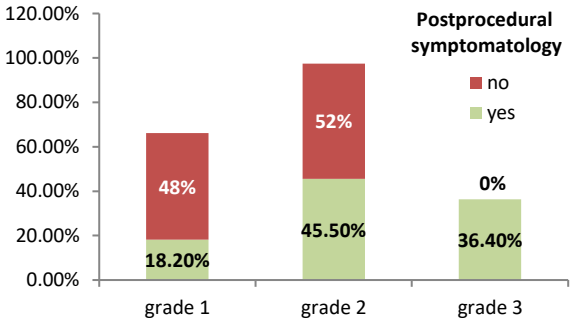
Resistant post-procedural symptoms may be influenced by the type of technique performed: resection of the diverticular pouch or just opening, and association of the myotomy or not. For the patients in the group, the resection of the diverticulum was performed only in the patients treated with open surgery.

The statistical association we observed comparing the percentage of resected Zenker's in patients with persistent postinterventional symptomatology (54.5%) compared to those without postinterventional symptomatology (4.0%) (p <0.01). (Table 1)

Of the 11 patients with postinterventional symptomatology, four patients had grade three diverticula. The correlation of the grade of the diverticulum with the persistence of the post-procedural symptomatology determined a statistical association to the dimension appreciated in degrees according to the Overbeeck classification.

The statistical association was found by correlating the persistence of postintervention symptomatology in patients with grade III diverticula (36.4%) compared with those without postinterventional symptomatology (0.0%) (p <0.01) (Table 1) (Figure 2)

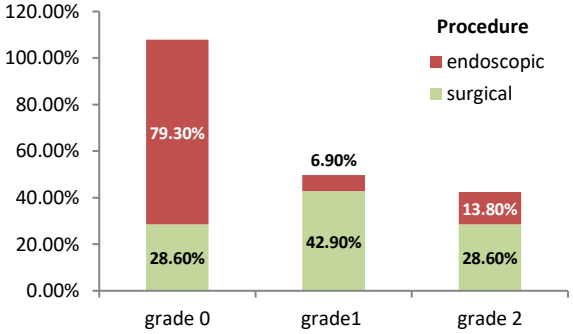
Figure 2: Distribution of patients with post-intervention symptomatology to the dimensional degree of the diverticulum.



In the post-procedural symptomatology dysphagia was the dominant symptom of 10/11 patients – 4 patients had dysphagia grade 1 and 6 patients dysphagia grade 2. The statistical association was observed between the percentage of patients with delayed dysphagia (p <0.01) and those with postinterventional symptomatology (90.9 %) compared to those without postinterventional symptomatology (4.0%). (Table 1) Persistent postinterventional symptomatology was mainly dysphagia.

Post-procedural persistent dysphagia was reported by 11 patients – 5 patients after surgical treatment and 6 after endoscopic treatment, 10 patients had a lower degree of dysphagia than at the initial presentation. Of the 5 patients surgically treated for 3 dysphagia persisted in the first postoperative month and did not require treatment, for the 2 patients with grade 2 dysphagia, one required re-intervention for a hematoma, and another one with esophageal fistula required adequate surgical treatment. We observed a statistical association between persistent dysphagia and surgical treatment (p <0.03), probably due to the small number of patients treated surgically. (Table 2) (Figure 3)

Figure 3: Distribution of patients according to the persistence of postintervention dysphagia to the surgical or endoscopic procedure type.



Tabel 2: Statistical correlation for redo procedure - statistical data analysis.

	Redo=Yes (N=7)	**Redo=No (N=29)**	**P_value (test)**
Sex=M	4/7 (57.1%)	17/29 (58.6%)	1.000000 (Fisher's Exact Test)
Grade			
1	0/7 (0.0%)	2/29 (6.9%)	
2	5/7 (71.4%)	15/29 (51.7%)	0.328714
3	2/7 (28.6%)	7/29 (24.1%)	(Likelihood Ratio)
4	0/7 (0.0%)	5/29 (17.2%)	
No Symptoms			
1	3/7 (42.9%)	8/29 (27.6%)	
2	1/7 (14.3%)	4/29 (13.8%)	
3	3/7 (42.9%)	12/29 (41.4%)	0.633852 (Likelihood Ratio)
4	0/7 (0.0%)	3/29 (10.3%)	
5	0/7 (0.0%)	2/29 (6.9%)	
Hospitalisation			
	6.29±6.7506 **3.00 [2.00, 14.00]**	3.55±2.9469 **2.00 [2.00, 3.50]**	0.360412 (Mann-Whitney Test)
Persistent dysphagia			
yes	**7/7 (100%)**	**4/29 (13.8%)**	**0.000040** (Fisher's Exact Test)
Persistent Dysphagia grade			
0	**0/7 (0.0%)**	**25/29 (86.2%)**	
1	2/7 (28.6%)	3/29 (10.3%)	**0.000009** (Likelihood Ratio)
2	**5/7 (71.4%)**	**1/29 (3.4%)**	
Sialorrhea			
Yes	2/7 (28.6%)	2/29 (6.9%)	0.162567 (Fisher's Exact Test)
Size			
	3.86±1.2149 4.00 [3.00, 5.00]	**2.93±1.2516** 3.00 [2.00, 3.50]	**0.086359** (Student T)
Size classif.			
1	1/7 (14.3%)	13/29 (44.8%)	
2	3/7 (42.9%)	15/29 (51.7%)	**0.023010** (Likelihood Ratio)
3	**3/7 (42.9%)**	**1/29 (3.4%)**	
Procedure			
surgical	2/7 (28.6%)	5/29 (17.2%)	0.601669
endoscopic	5/7 (71.4%)	24/29 (82.8%)	(Fisher's Exact Test)
Miotomy			
Yes	7/7 (100%)	28/29 (96.6%)	1.000000 (Fisher's Exact Test)
Pouch resection			
Yes	2/7 (28.6%)	5/29 (17.2%)	0.601669 (Fisher's Exact Test)
Intra op morbidity			
	1/7 (14.3%)	2/29 (6.9%)	0.488235 (Fisher's Exact Test)
Comorbidity			
yes/no	7/7 (100%)	21/29 (72.4%)	0.309298 (Fisher's Exact Test)
Comorbidities – No			
0	0/7 (0.0%)	8/29 (27.6%)	
1	3/7 (42.9%)	5/29 (17.2%)	
2	4/7 (57.1%)	7/29 (24.1%)	**0.033327** (Likelihood Ratio)
3	0/7 (0.0%)	8/29 (27.6%)	
4	0/7 (0.0%)	1/29 (3.4%)	

HTN	4/7 (57.1%)	15/29 (51.7%)	1.000000 (Fisher's Exact Test)
DM	0/7 (0.0%)	5/29 (17.2%)	0.558991 (Fisher's Exact Test)
Dyslipidemia	2/7 (28.6%)	12/29 (41.4%)	0.681030 (Fisher's Exact Test)
Other diverticula	1/7 (14.3%)	3/29 (10.3%)	1.000000 (Fisher's Exact Test)

Post-procedural sialorrhea was reported by four patients. It was not present as a unique symptom but it was always associated with persistent dysphagia. Post-procedural sialorrhea persisted in four patients.

Reintervention was necessary when the persistence of post-procedural symptoms was recorded for 7/11 patients. For the 5 patients treated endoscopically, who required reintervention for the persistence of the symptomatology, an average interval of 22.8 months was observed, while the reinterventions for the 2 patients treated by open surgery happened during the postoperative period. The statistical association was observed in patients who needed reintervention and those with postinterventional symptomatology (63.6%) ($p < 0.01$) (Table 1)

DISCUSSIONS

When comparing the technical and clinical results from the literature on the treatment of Zenker diverticulum, it should be critically emphasized that a direct comparison between studies and results may be inadequate, as the data are neither homogeneous nor standardized in regards of multiple variables. Noteworthy in this regard: symptom collection (dysphagia, dysphagia plus regurgitation, cumulative symptoms), symptom evaluation (objective scores for dysphagia, subjective grading of symptom relief and satisfaction), choice of one or another technique (sequentially, size of the diverticulum, clinical conditions, local policy), the definition of clinical success (total disappearance of symptoms, disappearance with improvement), success and extent of recurrences (after one or more sessions) and variable duration of follow-up (the shortest follow-up programs being reported after endoscopy) flexible, compared to the more recent series of patients treated transorally and with a historical cohort of patients treated surgically).

Patient follow-up included history taking, clinical examination, imaging investigations. Clinical criteria followed were persistence or recurrence of dysphagia and sialorrhea as with other similar studies. [13]

In patients in our group, we found the efficiency of endoscopic treatment of 96% and the whole group of 80%. Most cases published since 1995 demonstrate the efficacy and safety of flexible endoscopy, with clinical success rates between 56% to 100%. [14-15]

The success rate lower than 56% reported in a single series [10] is most likely because the clinical remission was evaluated according to the presence or absence of a group of symptoms and not only dysphagia.

The rate of recurrent symptoms was significantly higher in the surgical group than in the endoscopic group, results different from those reported in other studies where a 39% recurrence rate was found after endoscopic treatment and 0% recurrence in the patients who suffered surgery, probably due to the small number of patients treated surgically. [13]

In the studied group, the persistent postinterventional symptomatology was mainly represented by dysphagia, which is why we consider that in other studies, the efficacy of the treatment should be reported according to the improvement of this symptom. Data from the literature report clinical success increases to 84-100% when the unique series in which success is defined according to dysphagia is taken into account. [9, 11, 15]

As in our study and some other studies, treatment efficacy was reported after a single treatment session, [10, 16] while in other series the success rate was reported after several treatment sessions. [9, 17]

Unlike rigid staple endoscopy, the flexible septotomy technique for the Zenker diverticulum is neither univocal nor standardized. As already mentioned, different cutting techniques can be variably combined with different accessories. The optimal dividing technique is unknown because prospective randomized studies are lacking, and the choice is mainly based on the endoscopist's personal experience and preferences.

In evaluating the success rate of surgery, the most important factor is the subjective evaluation of the swallowing function performed by the patient. Both the primary and revision indications are guided by the patient's symptoms. [18]

Following rigid endoscopic or surgical treatment, persistent septum or residual pouch may still be present on barium

contrast examination. Persistent symptoms may be due to other swallowing abnormalities and/or inappropriate myotomy, without correlating the size of the residual pouch and the recurrence of the symptoms. There is a consensus in the literature that the evaluation of treatment efficacy and the need for further treatment must be determined by the patient's symptoms [19, 20] - which we agree with.

Some authors concluded that the presence of a residual diverticulum on an early postoperative esophagogram does not warrant early surgical revision, even when dysphagia persists. In many cases, there may be a slow process of gradual fibrosis of residual dysfunctional cricopharyngeal muscles or atrophy of the remaining diverticular sac over time. In addition, a normal postoperative esophagogram probably cannot predict a good and persistent outcome.

Some authors have reported that only 12.5% of patients with remaining diverticula on the postoperative contrast check develop a recurrence of Zenker's diverticulum. [18]

The approach of redo interventions has changed over time. If in older studies, there is a high percentage of recurrent surgical treatment, [21, 22] we are currently attending an endoscopic revision intervention, and in our study was noted that on a case of redo was necessary to use an endoscopic method after open resection of the Zenker diverticulum for the treatment of dysphagia in case of postoperative stenosis.

Other authors point out the need for classical intervention to resect the residual sac, which, even after a complete myotomy, can be a reason for the persistence of food retention mechanism. This may be the cause of symptoms in patients with prolonged retention of the contrast substance in the pharyngeal pouch and difficult passage into the esophagus, even after complete cricopharyngeal myotomy. [2, 3]

Other authors also suggest that an incomplete cricopharyngeal myotomy may result in higher recurrence rates associated with flexible endoscopy. Ideally, the diverticulum should be reduced to <1 cm. [24]

Incomplete cricopharyngeal myotomy may be visible on radiographs as a cricopharyngeal impression. Hypo-pharyngeal dysfunction during swallowing of the contrast substance may indirectly suggest incomplete myotomy or recurrent laryngeal nerve injury. On radiographs with contrast substance, pharyngeal dysfunction is suspected if we see contrast substance in the larynx or the tracheobronchial tree. Some authors consider that the persistence of the septum rather characterizes the endoscopic technique and does not predict the failure of the treatment, especially during the immediate postoperative period. [25]

Other authors have tried to evaluate the efficiency of the procedure through manometric studies. Ozgursoy and Salassa reported that a large diverticulum can cause obstruction of the esophageal passage and can lead to high pressure, even if the tone of the upper esophageal sphincter remains normal at manometry. Such a theory leads to the hypothesis that the weak wall of the hypopharynx may play an important role in the development of the diverticulum and leaving the weak adynamic segment of the esophagus creates a favorable environment for recurrence. In addition, the creation of a large cavity after diverticulostomy favors endoscopic techniques over open diverticulectomy on manometric evaluation, because, in a large cavity, small pressures are difficult to record, even if part of the septum remains and this causes dysphagia. [26, 27]

Some studies have shown that post-procedural soluble contrast studies do not correlate with symptoms following endoscopic therapy or relapses and that the second radiological examination, three months after surgery, presents the final functional results, with which we also agree. [8, 25] We also carried out new post-procedural or post-operative radiological studies to exclude a fistula on the seventh postoperative day and in the post-procedural monitoring of patients. Maybe a more accurate assessment of the contrast substance study could be done 3 months after the procedure.

For the patients in the group, we found that the duration of hospitalization in patients with postinterventional symptomatology and those without postinterventional symptomatology was significantly different. The average duration of hospitalization was 2 days for patients without post-procedural morbidity and 7 days for those with post-procedural morbidity comparable to the data in the literature. In our study, the duration of hospitalization of patients with open diverticulectomy was comparable with the data in the literature. [28-30]

The persistence of dysphagia in the 11 patients included in the group usually required reintervention; most of the patients with persistent symptomatology required a reintervention of the same type. Similar studies have also been reported in the literature, most patients who failed the procedure also underwent endoscopic reintervention. [31]

In the patients in the group with bulky diverticula of degree III or larger than 4 cm, the most frequent post-procedural reinterventions were required, 3 out of 4 patients, aspects encountered in other studies. Partial septotomy in the diverticula greater than 5 cm has been proposed by some surgeons, even if the myotomy fails to completely remove

the septum and may require more endoscopic interventions. [16, 32, 33]

According to recent literature, some authors recommend the endoscopic procedure for revision surgery as well. The great advantage compared to the open approach is that the endoscopic revision treatment is technically feasible and relatively easy, not more difficult than the primary endoscopic procedure, without an increase in risk. Furthermore, patient satisfaction was higher in those who underwent endoscopic revision surgery compared to those who underwent open surgery revision. [6, 18, 34]

Repeated procedures, in case of therapeutic failure or symptomatic relapse, can be easily and successfully performed by flexible or rigid endoscopy. An additional advantage is that repeated procedures for persistent or recurrent symptoms can be successfully achieved through a transoral (rigid or flexible) approach, while an open approach can be a major technical challenge. [5]

The data from the literature show recurrence rates of about 6% (range 0-22%), with the average recurrence rate of 5% compared to the classical approach, and according to data obtained in our study. [35]

The comorbidities of patients with diverticula included in the group also played a role in the emergence of the situations that required reintervention. [36]

Some authors consider that endoscopic findings tend to decrease over time and that incomplete septotomy is a significant factor for failure rate [37-40], an aspect we have also found.

CONCLUSIONS

Most of the time redo procedures were of the same type, the endoscopic iterative treatment being much easier than the surgical iterative treatment. Undoubtedly, endoscopic treatment has gained more and more confidence even in the field of revision interventions, considering that persistence or recurrence of dysphagia has as substrate an incomplete division of the septum requiring the same type of procedure. Redo procedures were necessary for patients with persistent dysphagia, bulky diverticula, and patients with a high number of comorbidities.

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EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine.
Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas.
Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

ETHICAL CONSIDERATIONS

Principles for Publication of Research Involving Human Subjects

Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013) (http://www.wma.net/en/30publications/10policies/b3/index.html).
It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

Registration of Clinical Trials

We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.
We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:
(1) Accessible to the public at no charge;
(2) Searchable by standard, electronic (Internet-based) methods;
(3) Open to all prospective registrants free of charge or at minimal cost;
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All articles, with the exception of Editorials, must contain an abstract of no more than 250 words. Abstracts for original articles should be formatted into subheadings, as detailed below. Titles must not be longer than 120 characters (including spaces).

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These are invited by the Editor-in-Chief or their delegated editor, and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure and cite up to 15 references, including the source article [this should be cited as Rom J Mil Med (year); (vol): [this issue].

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RJMM welcomes reviews of important topics across the scientific basis of medicine, and advances in clinical practice. Most published reviews are in response to editorial invitation, including thematically related “mini-series” of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

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The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as 'What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?' Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors' revised 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication', as presented at http://www.ICMJE.org/.

Spelling. The journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster's Collegiate Dictionary.

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The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

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The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

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As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

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Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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MINISTERUL APĂRĂRII NAȚIONALE

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dorind a răsplăti meritele

redacției pubilcației „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,

conferă ***Emblema de Onoare a Armatei României***
-cu însemn de pace-

Acordat în Bucureşti, 15 Noiembrie 2017

Ministrul apărării naţionale

Mihai-Viorel Fifor

Ordinul nr.M.P.3 din 15.11.2017
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