Founded 1897 • Vol. CXVIII

New series • No. 2/2015

Romanian Journal of Military Medicine

REVISTA DE MEDICINĂ MILITARĂ

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- *From liver biopsy to non-invasive markers in evaluating fibrosis in chronic liver disease*
- *Diabetes mellitus and colorectal cancer – a revealed connection*
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- *Case report – hepatocytolytic syndrome hiding mesenteric venous ischemia*
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Romanian Journal of Military Medicine (RJMM) is included in Romanian College of Physicians Medical Publications Index and credited with 5 CME credits. RJMM is recognized by CNCSIS in the category C.

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Romanian Journal of Military Medicine, vol. CXVIII, New Series, No 2/2015

ISSN 1222-5126

RJMM
Romanian Journal of Military Medicine

Founded 1897 • Vol. CXVIII
New Series • No. 2/2015

Edited by the Romanian Army Medical Directorate and Romanian Association of Military Physicians and Pharmacists.

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EDITORIAL

PancreaticFest 2015, annual EUS international workshop

Mariana Jinga, Vasile Balaban

Colonel MARIANA JINGA

Deputy Commander, Central Emergency Military Hospital, Bucharest
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Although neglected in the past, pancreatic pathology (comprising acute and chronic pancreatitis and the fearful pancreatic cancer) has been in the spotlight in the last decades both in gastroenterology and surgery. This growing interest for pancreatology has led to funding of innovative research, publishing of highly accessed papers, and development of tertiary centers, with solid teams of gastroenterologists, radiologists, pathologists and surgeons; this has also led to a need for better risk stratification and for improvement in both early diagnosis and treatment of pancreatic diseases.

While death rates for many other cancers are declining, mortality for pancreatic cancer is increasing. The incidence of acute pancreatitis is also on the rise, and the disabling symptoms of chronic pancreatitis can greatly alter quality of life and limit work.

Despite the fact that these pancreatic diseases carry significant burden for society and medical systems, there are still many unmet needs in managing patients suffering from these illnesses, especially in Romania – lack of awareness in primary care, few dedicated centers and difficult access to novel diagnostic and treatment methods, which are not reimbursed by policy-makers.

In this setting, the Internal Medicine and Gastroenterology Clinic at "Dr. Carol Davila" Central Military Emergency University Hospital has proved a special interest for the study and management of pancreatic pathology. Back in 1998, the hospital was the first in Romania to have a linear endoscopic ultrasound (EUS) system. A few years later, EUS guided fine needle aspiration was also performed in our clinic in national premiere. Since then, EUS has evolved into a therapeutic instrument, especially for the pancreas and biliary tree, and research to extend its indications is ongoing.

To promote the interest for pancreatology, our clinic has been hosting an annual EUS workshop, with a first edition in 1999, which has grown into a tradition in the fall of the last few years. Since 2013, the workshop is held together with the International Pancreatic Disease Course, coordinated by Professor Cristian Gheorghe. The "Pancreatic Fest" is organized by the Association for Pancreatic Pathology Romania (APPR), which is part of the European Pancreatic Club. Over the years, world renowned experts have lectured and performed live demonstrations at the combined pancreas meeting, setting high standards for Romanian doctors and motivating young gastroenterologists to get training in this field. This up-to-date meeting in pancreatology reunites more and more attendees each year, creating opportunities for exchange programs abroad, developing

multicenter research projects and promoting excellence care for patients.

This year's edition of the Pancreatic Fest, will take place on September 11-12th in Bucharest. The first day, dedicated to the EUS Workshop, will bring the participants complex cases, difficult techniques, tips and tricks, and cutting-edge EUS technology, all in HD live demo transmission from the Endoscopy Unit of the "Dr. Carol Davila" Central Military Emergency University Hospital. The second day will offer the participants a great educational programme, with state of the art lectures from international expert pancreatologists and interactive talks. We aim to bring together gastroenterologists, surgeons, pathologists, oncologists, radiologists, intensive care specialists and basic scientists, to create multidisciplinary teams with expertise in diagnosing and treating patients with pancreatic diseases. The message we are trying to convey is that partnering of specialties is the key to the successful management of these patients. We look forward to seeing you in Bucharest for a valuable scientific experience in the start of the fall!

REVIEW ARTICLE

Article received on May 12, 2015 and accepted for publishing on June 21 2015.

From liver biopsy to non-invasive markers in evaluating fibrosis in chronic liver disease

Cătălina Diaconu[1], Florentina Ioniță Radu[1,3], Mariana Jinga[1,2], Petruț Nuță[1], Săndica Bucurică[1,2], Bogdan Macadon[1], Mihăiță Pătrășescu[1], Andrada Popescu[1], Vasile Balaban[1,2], Laura Voicu[1], Daniel O. Costache[1], Raluca S. Costache[1,2]

Abstract: *Chronic liver disease is a late stage of progressive hepatic fibrosis. It consists of functional and structural disruptions in most chronic liver diseases. An accurate diagnosis allows us to establish the degree of fibrosis and the stage of the disease, the prognosis of the patient and to predict a treatment response. Despite the fact that liver biopsy is considered a gold standard, non-invasive methods for diagnosing liver fibrosis have gained more and more importance. Whether we talk about serum biomarkers or imagistic methods from transient elastography to 3-D magnetic resonance elastography, the question remains: are these useful or useless? Serum biomarkers represent blood components that can reflect liver histological changes, thus they can monitor the continuous process of fibrosis. These can be subcategorized in direct (that show extracellular matrix turnover) and indirect markers (that reflect disturbances in the hepatic function). However these markers alone are not as accurate in the staging of fibrosis, only help differentiate patients without or with low grade of fibrosis from those with significant fibrosis and cannot be considered alone in the diagnosis of liver fibrosis. Imagistic methods include: ultrasound-based transient elastography, magnetic resonance elastography (MRE), 2D-shear wave elastography, acoustic radiation impulse imaging (ARFI) and cross sectional imaging, the first being the most used. Using a combination of non-invasive tools allows us to diminish the number of patients in need of liver biopsy. However, the patient must always be informed of the advantages and disadvantages of each method and its limitations.*

Keywords: *fibrosis, biopsy, elastography, biomarkers*.

INTRODUCTION

Liver fibrosis remains an important problem worldwide. At first liver fibrosis was considered irreversible, but now scientists show that it is a dynamic process [1]. Advanced chronic liver disease, also known as cirrhosis is defined by the replacement of normal liver tissue with fibrotic one, leading to the disruption of the normal architecture of the liver and formation of regenerative nodules. It is usually considered to be irreversible, eventually leading to the need of liver transplantation. Many liver diseases can lead to cirrhosis. Most encountered causes are:

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viral hepatitis (mostly B and C hepatic virus), alcoholic liver disease and nonalcoholic liver disease, hemochromatosis. One must bear in mind less common causes of advanced end stage liver disease such as: autoimmune hepatitis, primary sclerosing cholangitis, primary and secondary biliary cirrhosis, Wilson disease, veno-occlusive disease, alpha-1 antitrypsin deficiency or right sided heart failure [2].

In normal hepatic tissue there is a balance between the production and degradation of extracellular matrix components. In liver fibrosis the balance is skewed in favor of the latter [3]. There is a high number of cells involved in the process of fibrogenesis. However, the hepatic stellate cell is the main factor involved. Chronic liver injury leads to the activation of hepatic stellate cells that transforms into myofibroblasts. These are contractile cells that secrete extracellular matrix components, pro-inflammatory citokines and chemokines. The first such protein produced in liver fibrosis is fibronectin, leading to the formation of excess collagen type 1. This process is cyclic since the production of extracellular matrix activates other stellate cells, which produce more proteins and finally change the hepatic architecture. Portal fibroblasts are also involved in the process of fibrosis, after stellate cells, tuning into myofibroflasts. Since these are closer to the portal tract they have a more important role in cholestatic injury from liver fibrosis, encountered in primary biliary cirrhosis and primary sclerosing cholangitis [4].

Liver biopsy is considered the golden standard in evaluating the degree of fibrosis. However it is limited by sampling errors, intra- and interobserver variability in histological interpretation and by the possible complications that may be encountered. There are many histologic systems that can appreciate fibrosis, but the one mostly used is semi-quantitative METAVIR score [5].

There are two categories of non-invasive methods in evaluating chronic liver disease: serologic panels of tests and radiologic tests. First ones quantify the levels of biomarkers in the serum samples and are more available. The latter consists of tests that measures liver stiffness using a “physical” approach. The two methods are complementary. Serum biomarkers are less accurate in detecting intermediate stages of fibrosis that in identifying cirrhosis, but present many advantages: high applicability, low costs, wide availability, and the possibility to be performed as outpatient [6]. Radiologic tests measure liver stiffness and are very useful in detecting cirrhosis. Combination between serum biomarkers and imagistic methods are very useful, slowly becoming a new golden standard in evaluating fibrosis in advanced liver disease [7].

Genetic risk factors for fibrosis are only now starting to gain importance. Literature shows that external factors such as alcohol and coinfections accelerate fibrogenesis. Studies now report variants in several genes that become associated with higher risk of fibrosis in chronic hepatitis C, NAFLD or AFLD [3].

LIVER BIOPSY

Considered the gold standard for the evaluation of tissue damage, including end-stage liver disease, liver biopsy is slowly falling behind non-invasive methods. Despite the fact that a high range of sensitive and accurate blood and imagistic tests can now be used to detect and even diagnose liver diseases, liver biopsy remains a valuable diagnosis tool, especially in difficult cases[8]. However the well-recognised limitations of this procedure have fuelled discussions regarding the correct diagnosis pathway in advanced chronic liver disease. The major problems of liver biopsy remain sampling and observational errors. Studies have shown that the larger the piece is, the lowest the risk of sampling errors is. The quality of the piece is in the length and the number of portal fields contained. A 20-25mm biopsy with more than 11 portal tracts is considered optimal specimen, even though some studies show that even a 15mm piece can be useful [3.9]. Even though we can obtain a large size biopsy, unfortunately it only reflects 1:50000 of the entire liver. Not only the length, but also the caliper of the biopsy needle is essential, a 16 gauge needle being the most appropriate to use [6]. Intra- or interobserver variability can also limit the usefulness of liver biopsy, leading to discrepancies in up to 20% of cases. Using histopathological scoring systems

diminished this problem [3].

Histologic scoring systems in chronic liver disease are of great importance to the progression of the disease, prognosis and helping us establish appropriate treatments. In *chronic hepatitis* the most encountered systems are: the Knodell score (histology activity index), METAVIR score, Ishak score (modified Knodell score). Other less used scoring systems are: the Scheurer system, the Batts-Ludwig system and Laennec system. *Nonalcoholic fatty liver disease* (NAFLD) can be divided in nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NAFLD can be evaluated by nonalcoholic fatty liver activity score (NAS). It sums up the degree of steatosis, lobular inflammation and ballooning of the liver cells, ranging from 0-8. Fibrosis is not included in NAS. *Primary biliary cirrhosis* uses two scoring systems: Scheuer or Ludwing, based on: the degree of fibrosis, bile duct loss, copper deposition and the degree of cholangitis and hepatitis. Even though liver biopsy in *primary sclerosing cholangitis* is uncommon, if it is performed then a histologic scoring system can be used. It allows us to define 4 stages based on the degree of inflammation and fibrosis [10].

Complication rates related to liver puncture range from 0.75% to 13.6% [3]. Minor complications after liver biopsy include transient discomfort, moderate pain and mild transient hypotension. Bleeding remains the most important complication of liver biopsy, along biliary peritonitis, perforation and pneumothorax [11]. Biopsy through transjugular route can reduce the risks in patients with coagulation disorders or advanced liver disease [9]. The number of complications is also diminished if we choose ultrasound-guided biopsies [12].

Liver biopsies should be performed as in-patient procedure since 60% of complications occur in the first 2 hours and more than 90% of complications are prone to happen during the first 24 hours[3,11].

SERUM BIOMARKERS

The "biological" approach of evaluating end stage liver disease is represented by serum biomarkers. Even though these parameters may not be liver-specific, they have been associated with fibrosis stage [6]. These so-called surrogate markers can be direct and indirect. The changes in the content of extracellular matrix are quantified by direct markers, whilst indirect markers show hepatic function disturbances. These markers can be used alone or combined, leading to different scores, which can be simple (AST to platelet ratio index (APRI), FIB-4 index, FORNS) or calculated after a formula (Fibrotest, Fibroscore, Fibromax) [3]. Simple scores might have lower diagnosis accuracy, but they have greater advantages: lower price, easy to calculate and widely available [6].

Indirect markers

AST to platelet ration index (APRI) is easy to calculate based on the platelet count and AST level. APRI was first used in patients with hepatitis C virus (HCV), human immunodeficiency virus and HCV coinfection and alcoholic liver disease [13,14].

Table 1. The correlation between AST to platelet ratio index and the probability of cirrhosis.

APRI <=0.3:	Unlikely cirrhosis or significant fibrosis
APRI >0.3 and <=0.5:	Unlikely cirrhosis, significant fibrosis possible
APRI >0.5 and <=1.5:	Significant fibrosis or cirrhosis possible
APRI >1.5 and <=2:	Likely significant fibrosis, cirrhosis possible
APRI >2:	Likely cirrhosis

FibroTest and *ActiTest* were first used in patients with hepatitis B and C. *Fibrotest* uses alpha-2-macroglobulin, gamma globulin, apolipoprotein A1, alpha-2-globulin (haptoglobin), gamma-glutamyl-transpeptidase and total bilirubin. It takes into consideration patients' age and sex [1]. Even though there are advantages of FibroTest such as: high availability and applicability (>95%), there are many drawbacks: lack of specificity for liver disease, interference with other comorbidities that can change the result (Gilbert's syndrome or hemolysis) or the difficulty to differentiate intermediate stages

of fibrosis [15]. ActiTest is a modified FibroTest that involves ALT, and evaluates both fibrosis and necroinflamatory activity of the liver [1].

Hepascore involves assessment of bilirubin, GGT, alpha-2-macroglobulin, hyaluronic acid, age and sex. A metaanalysis showed that Hepascore did not bring more information than FibroTest in patients with alcoholic liver disease. However, other studies showed its accuracy in predicting fibrosis in patients with chronic HC [1,9].

AST/ALT ratio in normal patients is around 0.8. Some studies show that a value over 1 suggests cirrhosis, but its utility is not certified [1].

Other indirect markers that are not worldwide used include: FIB-4 index, NAFLD fibrosis score (cutoff higher than 0.676 is associated with positive predictive value of fibrosis and a value under -1.455 is associated with a negative predictive value of 88 percent), PGA index (alcoholic liver disease with a detection range 66-72%), Forns (similar performance with APRI), BARD score (predictive in patients with NAFLD)[1,6].

Direct markers are used in forming panels to predict fibrosis, but none are available in clinical practice. (Table 2).

Table 2. Direct markers of fibrogenesis and fibrolysis

Matrix deposition	Procollagen I peptide
	Procollagen III peptide
	Type I collagen
	Type IV collagen
	YKL-40 (chondrex)
	Laminin
	Hyaluronic acid
Matrix degradation	MMP-2
	TIMP-1, -2
Cytokines	TGF-beta
	TGF-alpha
	PDGF

Serum biomarkers aren't useful in distinguishing between intermediate stages of fibrosis, but are more accurate in detecting fibrosis. Moreover careful consideration is entitled when talking about patients with HCV-HIV coinfection since false positive results can appear. For example: APRI, FIB-4, FibroTest (due to hyperbilirubinemia induced by atazanavir), Forns Index (related to GGT increase from nevirapine) and HepaScore. Despite the fact that bioseric markers can be used for detecting cirrhosis, their utility is reduced in diagnosing significant fibrosis [6]. Combination of different scores can be effective in avoiding biopsies, but a single surrogate marker cannot replace liver biopsy [3].

RADIOLOGIC TESTS

Radiologic tests represent the "physical" approach in evaluating fibrosis in chronic liver disease. The methods include: ultrasound-based elastography, magnetic resonance elastography (MRE), acoustic radiation force impulse imaging (ARFI), 2D-shear wave elastography (2D-SWE) and cross sectional imaging [3].

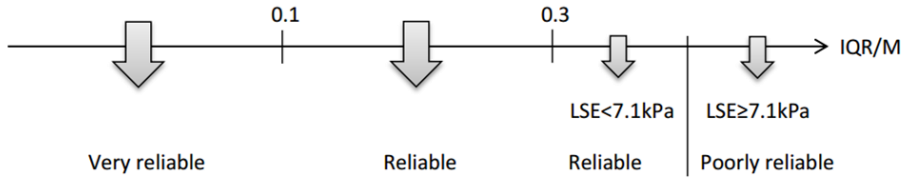
Trasient elastography was first evaluated as valid marker for fibrosis in a clinical study in 2002, therefore it remains rather new in the diagnostic pathway. It assesses the liver fibrosis by calculating the velocity of a low-frequency shear wave (50Hz) produced by a mechanical probe placed directly on the skin. The wave penetrates the liver tissue depending on the stiffness of the liver, correlating to the extent of liver fibrosis. The technique goes as following:

- the transductor is placed in contact with the skin with the tip covered in coupling gel;
- the position is in the 9^{th} to 11^{th} intercostal space (where liver biopsy is performed),
- we select an area of liver at a maximum of 6 cm deep, free of vessels;
- we gather 10 consecutive successful measurements. If one value is not valid, the entire set must be repeated [16].

There are several markers that evaluate if the result is valid: 10 consecutive valid results, a success rate (valid shots/total number of shots) above 60%, interquartile range lower than 30% of the median liver stiffness measurements, serum aminotransferase levels less than 5 times the normal value. Liver stiffness is expressed in kilo Pascal (kPa), ranging

from 1.5 to 75kPa. A three category system is used: very reliable (IQR/M≤0.10), reliable (0.10<IQR/M≤0.30 or IQR/M>0.30 and liver stiffness evaluation (LSE) <7.1kPa) and poorly reliable (IQR/M>0.30 and LSE≥7.1kPa) [16,17].

Figure 1. The accuracy of transient elastography is based on a system made from: interquartile range/median liver stiffness measurements (IQR/M) and LSE (liver stifness evaluation) [3].



The largest series report evaluating TE show that in 3.1% of cases the measurement was impossible and in 15.8% of cases the results were unreliable (mostly due to patient obesity). Therefore a new probe was developed: XL, 2.5MHZ transducer. This allows us to measure liver stiffness from 35 to 75mm depth [3,6]. In their study Myers et al concluded that in 276 patients with chronic liver disease (42% viral hepatitis, 46% NAFLD) and a BMI>28 kg/m^2, using an XL probe was more successful than using M probe. The number of unreliable results was lower when using XL probe (25%) than with M probe (50%). Stiffness values calculated with XL probe are lower by a median of 1.4kPa than when obtained with M probe [18,19].

Other limitations in using transient elastography are: narrow intercostal spaces, ascites, cardiac failure, high necroinflammatory activity (quantified by a high AST/ALT ratio), steatosis and cholestasis [20]. Excessive alcohol ingestion or food intake might modify the results of TE, therefore a fasting period prior to the investigation in recommended, 3-6 hours [6,21].

A special S probe was introduced for evaluating liver stiffness in children or patients with small intercostal space [22].

A meta-analysis of 50 studies comparing transient elastography and liver biopsy showed that we can choose an imagistic method in distinguishing cirrhosis from lesser degrees of fibrosis, but it is less reliable in detecting lower grades of fibrosis thus it has moderate negative predictive values. Moreover the study shows that transient elastography is better in diagnosing nonalchoholic steatohepatitis and alcohol-related disease than in chronic viral hepatitis. Transient elastography is useful in detecting cirrhosis with an optimal cut-off value of 13.01 kPa[3]. However one must take into consideration that the target population might have different characteristics and it should be pre-tested [6]. TE should not be used alone in evaluation on liver fibrosis in some patients, but if it predicts significant fibrosis there is no need for biopsy [3,4]

The performance of TE in hepatitis B and C is similar if we compare the two. When evaluating hepatitis e antigen (HBeAg-) negative patients with normal ALT values, non-invasive methods, especially TE could be used to estimate levels of HBV-DNA and identify patients in need of liver biopsy [6].

TE might be used in monitoring liver fibrosis: either after or during treatment or to evaluate the natural progression of the disease. A most discussed topic in the world of hepatology is the role of TE in predicting portal hypertension. A cut-off value of 27.5kPa can predict the presence esophageal varices and the need of endoscopy to evaluate the extent of the disease and the need to start primary prophylaxis with non-selective β-Blockers, whilst a cut-off value of 62.7kPa can suggest a high risk of esophageal bleeding. Studies show that a cut-off value >25kPa is associated with > 45-fold risk to develop hepatocellular carcinoma in patients known with viral hepatitis [3,23]

Shear wave elastography (SWE) is divided in: point shear wave elastography (pSWE), also known as acoustic radiation force impulse imaging (ARFI) and 2D-shear wave elastography (2D-SWE). ARFI excites

liver tissue by using short-duration acoustic pulses (~262µsec) that propagate shear waves and lead to localized µ-scale displacement in the tissue. The target zone (10x15mm) is set 10-20mm under the liver capsule. Ten consecutive valid measurements are required and the accuracy is evaluated with the interquartile range. The failure rate is lower when using ARFI to evaluate fibrosis than TE (2.9% vs 6.4%, p<0.001), especially in patients who suffer from obesity or associate ascites. Food intake, necroinflammatory activity and serum levels of aminotransferases might influence the results. ARFI is better in evaluating fibrosis in chronic hepatitis C than in chronic hepatitis B, NAFLD, HIV-HCV coinfection and other liver diseases. *2D-SWE* uses a combination of radiation force from focused ultrasonic beams and a very high frame rate ultrasound imaging sequence. Failure rate is lower than TE, especially in patients with ascites, but not in patients with obesity where XL probe is more accurate. However quality criteria in SWE are not yet well defined [6, 24].

Table 3. The correlation between the number of cases and SIRS criteria

Cirrhosis caused by:	Trasient elastography evaluation cut-off value (kPa)
Hepatitis C	13.01
Hepatitis B	11.7
Alcoholic fatty liver disease if drinking	22.7
Alcoholic fatty liver disease if abstinent	12.5
Biliary liver disease	17.9
Non-alcoholic fatty liver disease	10.3

Magnetic resonance elastography (MRE) involes placing a probe on the patient's back that emits low-frequency vibrations that pass through the liver and can be measured. With an optimal cut-off of 4.71 it can suggest cirrhosis with a sensitivity of 91% and a specificity of 81%[1]. Some studies show a better diagnostic accuracy and technical success than TE[25]. Moreover, MRE has the advantage of scanning the entire liver tissue, thus it cannot miss areas of fibrosis, hepatocellular carcinoma, focal liver lesions, and it is independent to an acoustic window and is operator independent [1]. However due to the costs and excess of time MRE requires, it is not used for routine clinical practice use [6].

Other imagistic methods such as computerized tomography are not currently in use for evaluating fibrosis in chronic liver disease, due to low sensitivity and specificity and high costs [3].

We now know that non-invasive tests have striking advantages against liver biopsy when talking about patient safety. Therefore by combining the serologic biomarkers with imagistic methods (preferably) or even different bio-markers we can limit using liver biopsy only in case of doubt or discordance between serological biomarkers and imagistic method. The combination of non-invasive markers allows rapid staging of the liver without the need for liver biopsy for hepatitis C. The current gold standard in hepatitis C is to associate TE and serum biomarkers [6].

In hepatitis B, TE is superior to serologic biomarkers and it is best used to detect the stage of fibrosis in patients with active viremia (DNA-HBV>2000 IU/ml) and normal ALT. TE is not indicated in hepatitis with very high levels of ALT >10 times the normal value. Liver biopsy should be used when in doubt [6].

Non-invasive methods in evaluating fibrosis in NAFLD have a lower accuracy compared to liver biopsy and in time patients might require histological confirmation. However, follow-up can be performed with serum biomarkers or TE once every 3 years to evaluate the progression of the disease [6].

Despite the fact that non-invasive tests cannot replace hepatic venous pressure gradient (HVPG) in evaluating portal hypertension or endoscopy that reveals clinical implication of portal hypertension (oesophageal varices), these might be useful in selecting patients that require these procedures. HVPG over 10mmHg is predictive of varices and decompensation, whilst values under 10 mmHg has a 90% negative predictive value for the development of clinical decompensation in 4 years. However measuring HVPG is both expensive and invasive therefore repeating measurements is impractical [4,6].

CONCLUSION

Non-invasive markers do not abolish the need for liver biopsy. They should be used to select the patients in need of liver biopsy more carefully.

Smart combinations of non-invasive markers can greatly reduce the number of liver biopsies performed and the risks associated, but when the diagnosis is unclear one must use liver biopsy.

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SYSTEMATIC REVIEW

Article received on March 10, 2015 and accepted for publishing on April 19 2015.

Diabetes mellitus and colorectal cancer – a revealed connection

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Abstract: *The burden of colorectal cancer (CRC) is increasing all over the world. The prevalence of diabetes mellitus is increasing. It is estimated that diabetes affects 387 million people worldwide. It is predicted that 552 million people worldwide will develop diabetes by 2030. A large pool of data indicate that DM increases by 2 fold the risk of CRC. This is the reason to firmly suggest the inclusion of DM in the criteria for CRC screening as an important measure to decrease the mortality of this ailment.*

INTRODUCTION

Type 2 diabetes mellitus and colorectal cancer are major causes of morbidity and mortality in the United States and other Western countries.

The burden of colorectal cancer (CRC) is increasing all over the world. It is recognized as the second cause of cancer death in USA and in Western Europe. Also, it is a third cause of cancer in men and a second cause of cancer in women. Every year there are 1,2 million new cases in the world. The highest incidence is encountered in Australia (75/100000), New Zeeland, North America and Europe[1]. In Romania the incidence has doubled from 1994 (13/100000) to 2005 (23/100000)[2]. The lifetime risk of CRC is 5%. 90% of cases appear after the age of 50; beneath this age threshold is rare encountered but the current trend indicates an increasing prevalence in young[3].

It is estimated that diabetes affects 387 million people worldwide. It is predicted that 552 million people worldwide will develop diabetes by 2030. In the United States, about 23.6 million people or 7.8% of adults have DM and by 2050, the number of people in the United States with diagnosed DM is estimated to grow to 48.3 million[4]. The prevalence of diabetes mellitus in Romania is 11.6% as PREDATTOR Study has concluded in 2014[5]. Even those figures are very high it is appreciated that almost one-third of diabetics is undiagnosed[6].

STUDIES THAT CONFIRM THE RELATION BETWEEN DM AND CRC

In 1910, Maynard[7] provided the earliest reports on the association between DM and cancer of any type. In 1984, Williams et al[8] published a retrospective study that documented a statistically significant, over 2-fold increase in the prevalence of DM in CRC

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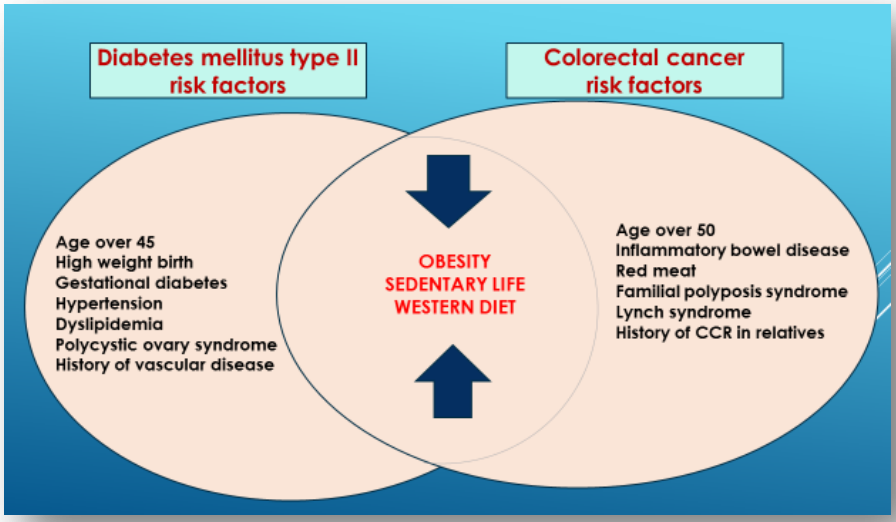
patients compared with age-matched controls. The search for a pathological explanation for this connection has led to the so-called hyperinsulinemia hypothesis. Giovannucci[9] hypothesized that insulin, an important growth factor, may at high serum concentrations increase the risk of CRC by promoting growth of colon tumors, and acting as a cell mitogen. Other proposed explanations for the increased risk include decreased bowel transit time and elevated fecal concentrations of bile acids[10]. Yang et al.[11] showed that insulin treatment may further elevate risk for CRC among patients with type 2 DM.

Figure 1: Risk factors for DM and CRC



Overall, studies indicate that diabetes risk increases twofold for liver, pancreatic, and endometrial cancer and 1.2- to 1.5-fold for colorectal, breast, and bladder cancer, but is associated with decreased risk for prostate cancer[12]. Diabetes is an independent risk factor for liver cancer. Cancer patients with diabetes relapse, and die, sooner than those without the disease.

Although proving a causal connection in observational human studies is uncertain and may take decades, preclinical research is revealing possible biochemical links between cancer and diabetes, which also share many risk factors.

The American College of Gastroenterology Guidelines for Colorectal Cancer Screening 2008 recommend that clinicians be aware of an increased risk of CRC in cigarette smokers and obese patients[13], but do not highlight the increased risk in patients with DM. Obesity and smoking are associated with the incidence of both type 2 DM and CRC[14]; thus, they also could be important positive confounders of the association between DM and CRC.

In July 2010, the American Cancer Society and the American Diabetes Association released a consensus report that reviewed the science behind the link and evidence for whether diabetes treatments influence cancer risk or prognosis. Published in the July August 2010 *CA: A Cancer Journal of Clinicians*, the report recommended regular cancer screening for diabetics. Preliminary evidence that metformin is associated with lower cancer risk, and exogenous insulin with higher risk[12].

Diabetes is characterized by high levels of both insulin and blood sugar, and both promote cancer cell proliferation in preclinical models In a Nov. 29, 2011, *British Journal of Cancer* study, he examined levels of the two in nearly 5,000 women from the Women's Health Initiative over 12 years. Elevated glucose levels were associated with a nearly doubled risk of colorectal cancer[12].

A recent meta-analysis on 41 studies associated DM with an increased incidence of colorectal cancer

[relative risk, 1.27; 95% confidence interval (CI), 1.21–1.34][15]. No associations between DM and colon cancer were evident among African Americans[16]. A large-scale, population-based study in Japan suggested that diabetic men carried a higher risk for colorectal cancer than diabetic women did. The incidence of colorectal cancer was significantly higher in DM patients than in control patients (HR, 2.10; 95% CI, 1.82–2.42. The incidence of colorectal cancer is higher in males than in females (adjusted HR, 1.34; 95% CI, 1.18–1.53)[17].

CRC risk was higher among diabetics with longer duration of disease[18].

Metanalysis [19] of 15 studies (six case – control and nine cohort studies), including 2 593 935 participants, found that diabetes was associated with an increased risk of colorectal cancer, compared with no diabetes (summary RR of colorectal cancer incidence = 1.30, 95% CI = 1.20 to 1.40), without heterogeneity between studies (*P* heterogeneity = 0.21). The association between diabetes and colorectal cancer incidence did not differ statistically significantly by sex (summary RR among women = 1.33, 95% CI = 1.23 to 1.44; summary RR among men = 1.29, 95% CI = 1.15 to 1.44; *P* heterogeneity = .26) or by cancer subsite. Diabetes was positively associated with colorectal cancer mortality (summary RR = 1.26, 95% CI = 1.05 to 1.50), but there was evidence for heterogeneity between studies (*P* heterogeneity = 0.04). Findings from this meta-analysis indicate that individuals with diabetes have an approximately 30% increased relative risk of developing colorectal cancer compared with nondiabetic individuals.

Risk factors such as obesity, physical inactivity, smoking, Western diet and metabolic syndrome are common in both DM and colorectal cancer patients.

THE PROBLEM OF CONFOUNDERS AS RISK FACTORS

The first metaanalysis to quantitatively assess the effect of certain potentially important confounding variables including obesity, smoking, and physical exercise was published in 2008. It showed a statistically significant association between DM and risks of colon cancer and rectal cancer separately after controlling for obesity, smoking, and physical exercise. The association of DM and cancer risk was stronger for colon cancer than for rectal cancer (summary RR 1.38, 95 % CI 1.26 – 1.51 vs. summary RR 1.20, 95 % CI 1.09–1.31, respectively). This difference may be because the proximal colon, distal colon, and rectum have different embryological origins[20].

The positive association of DM with colon cancer and rectal cancer risk did not decrease when the metaanalysis was limited to studies that controlled for smoking and BMI (summary RR for colon 1.34, 95 % CI 1.17–1.52, and summary RR for rectum 1.28, 95% CI 1.06–1.54). This suggests that the confounding effect of obesity and smoking is relatively weak, and that DM appears to be an independent risk factor for colon cancer and rectal cancer.

Furthermore, as a recent study indicated, persons with DM and CRC may be at increased risk for CRC recurrence, non-response to chemo and radiotherapy treatment, and treatment-related complications. CRC deaths may be reduced through CRC screening programs.[21]

PROPOSED MECHANISMS

Several mechanisms have been proposed to explain the potential relationship between DM and colorectal cancer. Hyperinsulinemia, insulin-like growth factor-1, glucagon-like peptide-1 and the relative binding proteins each play an important role in metabolism, cell growth, proliferation and the regulation of the apoptotic process in colon cells[22].

Mechanistically, insulin stimulates cell proliferation through two pathways. One pathway involves direct binding of insulin to insulin or insulin-like growth factor-1 (IGF-1) receptors, and the other pathway is via inhibition of IGF binding proteins and the resultant increase in IGF-1 availability to the IGF receptor. The IGF system is a potent growth regulator closely linked with carcinogenesis[23]. These data support the notion that IGF-1 has a role in the biological pathway of colorectal neoplasia, beginning at the adenoma stage.

CONCLUSION

The increasing prevalence of DM may, significantly, be responsible for the increasing prevalence of CRC and its mortality. A great pool of data may suggest a need for more intensive CRC screening program in patients with type 2 DM, especially those who receive chronic insulin therapy.

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ORIGINAL ARTICLES

Article received on March 7, 2015 and accepted for publishing on April 19 2015.

Culprits in non-celiac gluten-sensitivity

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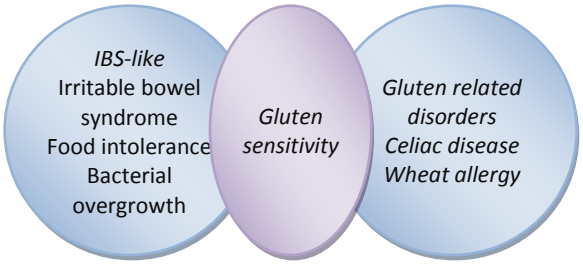
INTRODUCTION

The growing interest for gluten-free diet (GFD) in the last decades has lead to a significant number of people going for this diet, in the absence of a diagnosis of celiac disease (CD). Owing to the perception that dietary gluten could be responsible for some of the symptoms in patients with irritable bowel syndrome (IBS)[1,2,3] (*Figure 1*), a new concept emerged, that of non-celiac gluten-sensitivity (NCGS), as it was named in an international consensus report[4]. NCGS describes the individuals whose symptoms get better on a GFD, in the absence of CD or wheat allergy[5]. The first reports about this condition date back to the late 1970s (two case reports) and the early 1980s (the Birmingham study)[6,7,8], and recently, some authors consider more appropriately to term it "people who avoid gluten"[9]. Biesiekierski et al.[10] were the first to publish a randomized clinical trial that proved the existence of NCGS; confirmation later came from the italian study of Di Sabatino[11], who showed in a randomized, placebo-controlled, cross-over design, a small, but statistically significant, alteration of symptoms in NCGS patients when they were gluten challenged.

The main issues of NCGS are[12,13,14]:

1. most of the times, NCGS is self-diagnosed of diagnosed by alternative health care practitioners
2. people who decide to go gluten-free rarely exclude CD before starting the diet, and when they address the doctor for evaluation, alternative diagnoses such as bacterial overgrowth or food intolerances (lactose, fructose) are found
3. aproximately one in four people with NCGS have persistent symptoms despite dietary gluten restriction

Figure 1: Spectrum of IBS-like syndromes (modified after Verdu AF, Am J Gastro 2009)



The prevalence of NCGS is variable, with reported values between 0.5 and 13%[15]. It is estimated that nowadays more people are following a GFD after a self-diagnosis of gluten-sensitivity than patients with CD[16]. Unlike the other gluten-related disorders, the diagnosis of NCGS is purely clinical, considering that there is no specific biomarker or characteristic histopathological changes on enterobiopsy. A diagnostic algorithm was recently proposed, which includes proof of both gluten responsiveness and

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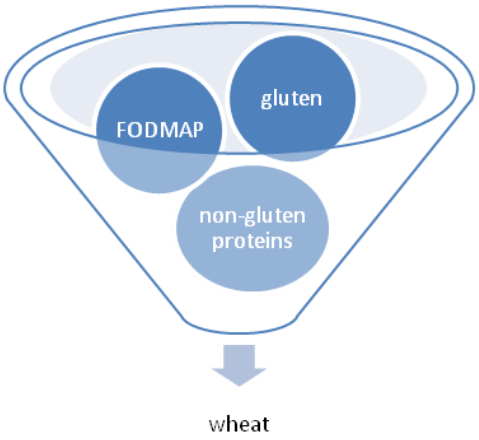
relapse on gluten challenge (Salerno criteria)[17].

ROLE OF GLUTEN AND OTHER TRIGGERS IN NCGS

The pathogenesis of NCGS is not completeley understood. The name NCGS implies that gluten is the culprit for the symptoms associated with this condition. However, recently there has been some questioning about gluten being the real trigger in NCGS, while considering other components which are also found in the cereals avoided in the GFD[18] – Figure 2.

Figure 2. Wheat components as possible triggers for NCGS



In this setting a "gluten conspiracy"[19] was described, which comprises two main ideas: if wheat is causing the symptoms, it must be the gluten in wheat responsible for them, and if the GFD improves the symptoms, this benefit is due to withdrawal of gluten. The pitfall of proving this misconception, that gluten is not the (only) one responsible, is that most of the challenges in clinical trials are performed with wheat (slices of bread, wheat flour capsules), and wheat contains not only gluten, but non-gluten proteins and non-protein components such as FODMAPs.

Among the possible non-gluten triggers, FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) and α-amylase/trypsin inhibitors (ATIs) have been studied in the literature.

The wheat kernel contains many components, among which the following are of interst for NCGS:
i) Proteins
- Storage proteins – represented by gluten (glutenins and prolamins – gliadin in wheat, and the equivalents secalin in barley and hordein in rye)
- Metabolic proteins – enzymes, enzyme inhibitors (eg. ATI – amylase-trypsin inhibitor), lectins (eg. wheat germ aglutinin – WGA)

ii) *Carbohydrates*
- Oligosaccharides, lactose, fructose, polyols (sorbitol, mannitol) – they osmotically draw water and are easly fermented by colonic bacteria (leading to gas formation in the gut lumen), which clinically translates into diarrhea, bloating and flatulence

FODMAPs

FODMAPs, an acronym for some osmotically-active, easily fermented carbohidrates, which are found also in gluten-containing cereals, have been described as a possible culprit of digestive symptoms in patients with NCGS[20,21,22,23]. A diet with reduced intake of FODMAPs (low-FODMAP), which has proven beneficial for IBS[24], has also been theoretized as being useful for NCGS patients.

In 2011, Biesiekierski showed that among cereal foods there is an overlap between the gluten-free and low-FODMAP ones, which could represent a confounder regarding the real culprit in NCGS: thus, the misconception that gluten is responsible for the digestive symptoms could be in fact explained by the presence of FODMAPs in the diet[25].

Another study performed by Biesiekierski on 37 NCGS patients (normal duodenal histology, negative HLA DQ 2/8) revealed that after 2 weeks on low-FODMAP (which lead to a significant relief of symptoms), there were no specific or dose-dependent effects upon dietary challenge in 3 randomized groups – high gluten (16 g gluten/day), low gluten (2 g gluten/day + 14 g whey protein/day) or control (16 g whey protein/day) diet for 1 week[26]. Gluten-specific effects were observed in only 3 out of 37 patients (8.1%). After a washout period of at least 2 weeks, 22 of the 37 patients crossed over to 3 groups which were given gluten (16 g/day), whey (16 g/day) or control (no additional protein) diet for 3 days. After the 3-day rechallenge, there were no differences between the three groups. Moreover, none of the 3 individuals

who had a previous gluten-specific response in the first part of the trial reported such a response upon crossover. However, this short exposure to gluten in the second part of the study was associated with higher depression scores, which could explain why NCGS patients feel better on a GFD despite the persistence of gastrointestinal symptoms.

In conclusion, this study proved that gluten had no effect in patients with NCGS placed on low FODMAPs diets, and these carbohydrates could be the true cause of symptoms. Thus, we can state that people who eat gluten-free could be in fact sensitive to FODMAP and not to gluten, and the benefits of the GFD could be explained by its overlap with the low-FODMAP diet.

Among the limits of this trial, we should consider the possible nocebo effect on reintroduction of gluten/whey in the study groups.

The questionable role of gluten in NCGS is also supported by the double blind, cross-over trial of Zanini, who showed that gluten challenge induces symptom recurrence in only one third of NCGS patients[27].

Amylase-trypsin inhibitors (ATIs)

Beside FODMAPs, Schuppan's group identified some non-gluten proteins as a possible trigger for NCGS, specifically amylase-trypsin inhibitors (ATIs)[28,29]. These have been shown to induce intestinal inflammation by activation of TLR4 (toll-like receptor 4), effect limited to gluten-containing cereals only (wheat,barley, rye)[18].

In addition to ATIs, other non-gluten proteins such as WGA (wheat germ aglutinin) are considered as possible triggers for NCGS-like symptoms[30]. Current data has shown that these lectins can increase intestinal permeability, through mechanisms still unknown.[31]

CONCLUSIONS

Current knowledge suggests that the symptomatic benefit of a GFD in NCGS patients could be in fact not the consequence of gluten elimination, but avoidance of other compounds found in cereals such as FODMAPs or ATIs. Considering the evidences that question the causative role of gluten in NCGS, some authors have proposed renaming of this condition to non-celiac wheat sensitivity[32].

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ORIGINAL ARTICLES

Article received on March 17, 2015 and accepted for publishing on April 19 2015.

Diagnostic and treatment difficulties in insulinomas

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Abstract: *Background: Neuroendocrine tumors of the pancreas (NTP) comprise a unique and relatively rare group of tumors, of which gastrinoma and insulinoma are the most common types. Insulinomas tend to be small, solitary and benign, with surgical resection curable in most cases.*
Introduction: Insulinomas are localized preoperatively using conventional imaging studies as transabdominal ultrasonography (US), computed tomography (CT), and/or magnetic resonance imaging (MRI).
Purpose: Endoscopic ultrasound (EUS) is a valuable tool in the diagnosis of insulinomas.
Goals & methods: We performed a retrospective study on 21 patients with insulinoma (6 male and 15 female, 25 to 73 years of age), who were hospitalized and operated on between 2003 and 2012 at "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest.
Results: US view was positive in 10% of patients (2 of 20), that presented proximal location. The sensitivity of CT was unsatisfactory, 21.05% (4 positive results of 19). CT failed to detect liver metastases, but identified nodal metastasis in one patient. MRI was performed in 18 patients and was diagnostic in 11 of them, recording a detection sensitivity of 61.11%, including infra-centimetric tumor size. EUS has a high resolution which allows detection of lesions with very small diameter is safe and minimally invasive. EUS was performed in all patients, being able to identify formations in 17, was inconclusive in 3, showing a diagnosis sensitivity of 81%. Liver metastases were demonstrated in 3 patients, one by US and all 3 by MRI.
Conclusions:
- CT with intravenous iodinated contrast agent had a poor sensitivity in detecting the primary tumors, was insensitive in detecting liver metastases, but showed metastases in lymph nodes.
- MRI has higher sensitivity than CT in detecting primary tumors, including insulinomas with infracentimetric size, and is the imaging test of choice for possible liver metastases.
- EUS is the preoperative imaging test of choice.

Keywords: *insulinoma, neuroendocrine tumors of the pancreas, endoscopic ultrasound (EUS), preoperative localization*

INTRODUCTION

Neuroendocrine tumors of the pancreas (NTP) are rare, but increasingly recognized entities. Tumors may be functional or nonfunctional, which are frequently detected incidentally or with symptoms related to mass effect of the tumor or its metastases (1). NTP have been considered rare, with an estimated incidence of less than 1 per 100000 individuals (2). Insulinoma is the most common

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neuroendocrine tumor of the pancreas and cause of hypoglycaemia related to endogenous hyperinsulinism with an annual incidence of four in every 1 million persons (3, 4, 5). As many as 90% of insulinomas have been reported to be benign, 90% are solitary, >90% occur in intrapancreatic sites, and 90% are < 2 cm in diameter. Regarding the location of pancreatic tumors, 50% are in the head, 25% in the tail, and 25% caudally or permeates the entire pancreas (5, 6).

Most of these tumors occur sporadically, but they can also be associated with multiple endocrine neoplasia (MEN)-1 syndrome. Approximately 4% of patients with insulinoma have MEN-1 (3, 4, 6). In sporadic forms, the highest incidence is considered in the age group 40-60 years (mean age 50 years), with a slight predominance of females (sex ratio 3:2 for women). (3). The diagnosis of insulinoma can be established by determining plasma proinsulin, insulin, C-peptide, and glucose levels, which are usually performed during a 72-hour fast (4). Insulinomas classically present with "Whipple's triad:" a combination of symptoms of hypoglycaemia, inappropriately high insulin levels with associated documented blood glucose levels of <50 mg/dL, and symptom relief with administration of glucose (2).

Non-invasive imaging procedures, such as CT and MRI, are used when a diagnosis of insulinoma has been made to localize the source of pathological insulin secretion. Invasive modalities, such as endoscopic ultrasound (EUS) and arterial stimulation venous sampling, are highly accurate in the preoperative localization of insulinomas and have frequently been shown to be superior to noninvasive localization techniques. Topographic preoperative diagnosis is difficult due to small tumor size; EUS is the most sensitive diagnostic method for this purpose. High resolution of EUS allows detection of lesions with very small diameter, is safe and minimally invasive (5, 7, 8). In the table below, we are presenting data from studies showing the accuracy of imaging methods in diagnosing insulinomas (Table 1).

Table 1. Accuracy of imaging methods in diagnosing insulinomas (5, 9, 10, 11, 12, 13, 14, 15, 16)

	US	CT scan	MRI	EUS	Intraoperative US	Intraoperative palpation
Pitre J, 1996				90%		
Angeli E, 1997	79.3%	44.8%	65.5%			
Ardengh JC, 2000		16.7%		83.3%		
Machado MCC, 2001	30%	25%	17%	27%		
Mirallié E, 2002				85%		
Jyotsna VP, 2006		57-83%	31%		92%	76%
Sotoudehmanesh R, 2007				89.5%		
Joseph AJ, 2013		69%		89%		
Takehiro O, 2013		33-64%	40-90%		80-100%	75-95%

There are still great difficulties in correct positive and topographic diagnosis of insulinomas. Advances in the pre-and intraoperative diagnosis allow precise localization and excision of the tumors, avoiding blind pancreatic resections (5, 17). Objectives of treatment are: symptomatic treatment of the tumor hypersecretion syndrome and surgical treatment which consists in enucleation of benign tumor or extensive resections. The surgery may use laparoscopic or open techniques. It is essential to perform intraoperative ultrasound, which will have an important role in choosing the optimal resection technique. Enucleation is the excision method of choice, whenever it is technically possible. Currently, resection of insulinomas can be successful with the techniques of minimally invasive surgery - laparoscopy or robotics, combined with intraoperative ultrasound performance. Medical treatment addresses to control symptoms or complications, for surgery preparation or as palliative treatment in cases where it can be done. When technically feasible, tumor enucleation is the

procedure of choice; however, a more formal resection may be necessary for certain tumors (3, 5, 7, 14, 18, 19).

GOALS

Given the low incidence of pancreatic neuroendocrine tumors and the relatively limited medical experience regarding this group of tumors, there is concern in the medical world to find methods of diagnosis and appropriate treatment.

This study aims to identify characteristic features of pancreatic neuroendocrine tumors, especially of insulinoma, the most frequently diagnosed of them. We analyzed in terms of demographics, clinical, laboratory, imaging, pathology and treatment 21 cases of pancreatic insulinoma, all diagnosed and treated at "Dr. Carol Davila" Central Military Emergency University Hospital", Bucharest, highlighting the best diagnostic and therapeutic attitude and the comparison with literature data.

We will try to determine which are most sensitive and specific methods applied at this time to establish early diagnosis and what treatment is recommended considering the particular features of the disease, the low incidence, appropriate index of suspicion, non-specificity of symptoms with often delayed diagnosis, involving a complex team (endocrinology, internal medicine, gastroenterology, oncology, intensive care, oncology, surgery) and the difficulties of this pathology.

MATERIAL AND METHODS

This article aims to develop a retrospective study to track the best diagnosis and treatment for insulinomas. In the period 25.01.2003 – 26.11.2012, 21 patients diagnosed with insulinoma were hospitalized and/or operated at the "Dr. Carol Davila" Central Military Emergency University Hospital in Bucharest. Medical records of patients with insulinoma seen in the period 2003-2012 were retrieved from the hospital's medical records department. From these, data on clinical and diagnostic features, localization and surgical outcome were extracted. All mathematical analysis was performed using Microsoft Excel 2007.

The study analyzes the following data: epidemiology (distribution by sex and age), clinical (symptoms), laboratory (laboratory diagnosis, topography, location, locoregional extension and/or distant metastases), histological (macroscopic, microscopic and immunohistochemical) and treatment (surgery, medical), postoperative evolution, complications and prognosis. Features of patients were highlighted and analyzed by comparison with the data currently available in the literature. Biological, imaging and histological methods of diagnosis and techniques and results of surgical treatment and care were analyzed in particular.

RESULTS

Over a period of nine years, between January 2003 and November 2012, 21 patients diagnosed with pancreatic insulinoma were admitted to "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest.

Gender distribution was 15 women (71.4%) and 6 men (28.6%) and sex ratio women: men of 2.5:1. A particular case was the association with Wermer syndrome (MEN I) found in a patient of 36 years, who was hospitalized for symptoms of insulin hypersecretion. The mean age at diagnosis of insulinoma was 46.66 years (25-73 years), being lower for women than men (41.06 vs. 53.66). Distribution by age showed a peak between 40 and 49 years.

Positive diagnosis is suggested by clinical manifestations. In 1938, Whipple described a triad with a high specificity: 1) neurological crises that occur on an empty stomach or after exercise 2) decrease in blood glucose below 50 mg% during the crisis, 3) disappearance of neuropsychiatric disorders following administration of glucose orally or intravenously.

The diagnosis is based on simultaneous measurement of blood glucose and insulin levels (5, 6, 7). In our cases, positive diagnosis was suggested by Whipple triad. All patients had presented with fasting hypoglycemia and fulfilled the triad to suspect a diagnosis of insulinoma.

CLINICAL PRESENTATION

Patients had various associations of symptoms, shown after prolonged overnight fasting and physical activity or stress. The fact that the symptoms occur early in the morning, on waking, and disappear after ingestion of carbohydrates or glucose administration oriented the diagnosis (7). Delays in the diagnosis of insulinoma are common because the symptoms usually precede detection of a tumor and there may be misattribution of the symptoms to psychiatric, cardiac, or neurological disorders and, on average, it takes up to 2 years before the correct diagnosis is made (5, 7, 8). Neuroglicopenic symptoms delayed diagnosis in 8 patients (38%), as they were hospitalized in the neurology and psychiatry wards and mistreated. Of them, four were diagnosed with epilepsy and received anticonvulsant treatment, and the others were labeled with neurological disorders, anxiety disorders or thyroid dysfunction. Finally, the diagnosis was correct, the time period between onset of symptoms and diagnosis ranging from several days to 10 years. The symptomatic evolution of the disease before diagnosis had a mean of 25.14 months. More than half of patients in the study (61.9%) were diagnosed as having insulinoma after less than a year of evolution.

Table 2. Frequency of symptoms and signs that patients presented with in the study

Symptoms	Number of patients (%)
Preprandial hypoglycaemia	21 (100)
Loss of consciousness	13 (62)
Dizziness	12 (57)
Diaphoresis	12 (57)
Tremor	10 (48)
Obesity	8 (38)
Fatigue / asthenia	8 (38)
Increased appetite / hunger	7 (33)
Palpitations	7 (33)
Headache	6 (28)
Loss of balance	6 (28)
Diplopia, blurred vision	6 (28)
Seizures	5 (24)
Extremity paresthesia	5 (24)
Impaired memory or concentration	5 (24)
Dysarthria	5 (24)
Confusion	4 (19)
Abnormal behavior	4 (19)
Anxiety, irritability, psychomotor agitation	4 (19)
Difficulty in waking up in the morning	4 (19)
Amnesia	3 (14)
Hypoglycemic coma	3 (14)
Temporo-spatial disorientation disorder	2 (9)
Affective disorder	2 (9)
Distal neuropathy associated with hypoglycaemia	1 (5)

Positive biological diagnosis

Insulinomas are the most common cause of hypoglycemia resulting from endogenous hyperinsulinism. B cell insulin secretion does not decrease in the presence of hypoglycemia, and so plasma insulin greater than or equal to 3µU/ml along with a plasma

glucose less than or equal to 50 mg/dL suggest an insulinoma. The diagnosis is made by simultaneous measurements of blood sugar and insulin levels in the blood (5).

The 72-h fasting test is a demonstration of Whipple's triad of symptoms, which is considered as the gold standard for the diagnosis. This test is conducted under supervised conditions, which requires hospitalisation of the patient. During the fasting period, the patient is allowed to drink calorie-free fluids and physical activity is encouraged. Blood glucose should be measured every 6 hours until it reduces to 60 mg/dL or less, when the interval is reduced to every 1–2 h. The fast is terminated when the plasma glucose level is 45mg/dl or less and the patient has symptoms and signs of hypoglycemia.

When symptoms of hypoglycemia appear, blood should be sampled for measurement of glucose, insulin, C-peptide, and proinsulin. Insulinoma is diagnosed when the following criteria are fulfilled: plasma concentrations of glucose less than 55 mg/dL (3.0 mmol/L), insulin of at least 3.0 µU/mL (18 pmol/L), C-peptide of at least 0.6 ng/mL (0.2 nmol/L), proinsulin of at least 5.0 pmol/L. Some authors described modified fasting test where duration of the fasting period is reduced to 48 h (3, 5, 7, 8, 21).

Glycemia during the crisis was on average 30.37 mg/dl (range 20-45 mg/dl). Plasma insulin measured was increased, the average being 25.9 µU/ml (6 to 79.2 µU/ml). A supervised fasting test was performed in 11 patients and was positive in 10 of them. Median time to a positive test was 15.5 hours (4-28 hours). In 3 patients (30%) the test was positive in the first 10 hours (after 4 hours fastest in 1 patient), in 6 patients (60%) was positive within 24 hours and in all patients (100%) was positive within 48 hours. No patient passed the boundary of 48 hours.

Differential diagnosis

Hypoglycemia due to excessive endogenous insulin secretion can be caused by a primary pancreatic beta cell disorder including an insulinoma, beta cell hyperplasia or nesidioblastosis (presence of diffuse microadenomatosis, in which multiple small non-encapsulated tumors or nodules are distributed throughout the pancreas), a beta cell secretagogue including a sulphonylurea or a beta cell stimulating antibody, or an antibody to insulin. The differential diagnosis of hypoglycaemia includes hormonal deficiencies, hepatic insufficiency, exogenous hyperinsulinism, medication, drugs and enzyme defects. Occasionally differentiating insulinoma from these other causes of hypoglycaemia can be quite difficult (7).

Differentiation for nondiabetic-associated hypoglycemia is broad and includes insulinoma, drugs, hormone deficiencies, and critical illness. For stratification, the causes of hypoglycemia can be divided into those associated with diabetes mellitus and those who do not suffer from this condition, as the pathophysiology of each is distinct. Drugs are the most common cause of hypoglycemia (antidiabetic agents – insulin, sulfonylurea and meglitinides, salicylates, quinine derivatives, disopyramide, pentamidine, ethanol). Hypoglycemia is not an infrequent finding in the critically ill patient and may be related to sepsis, hepatic or renal failure, or to general malnourishment. Hepatic or renal failure, deficiency of cortisol and/or growth hormone can lead to hypoglycemia, leads to an inability to maintain adequate fasting glucose levels despite adaptive renal gluconeogenesis. Hypoglycemia associated with endogenous hyperinsulinaemia is very rare. Differential diagnosis of fasting hypoglycemia in adults should also include nonislet tumor (mesenchymal tumors, hepatocellular carcinoma or hematological malignancies) and autoimmune hypoglycemia (insulin receptor autoantibodies, insulin autoantibodies) (22, 23, 24, 25).

Topographic diagnosis

Transabdominal ultrasonography (US) was performed in 20 of the 21 patients and was positive in two of them (10%), which shows no accuracy of topographic diagnosis. A positive US result was obtained in a patient with proximal insulinoma (the pancreatic region most accessible for abdominal ultrasound exploration) – at the pancreatic head-body junction, and the other in a patient with caudally located tumor, but large (63/43 mm).

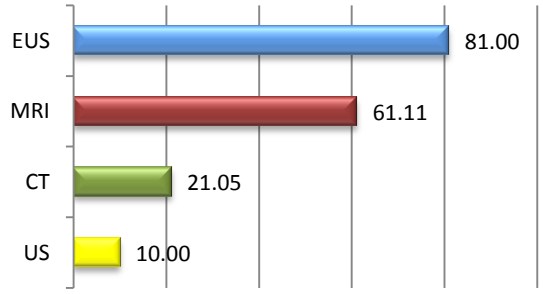
In one patient with a history of metastatic insulinoma, previously operated, followed by a second surgical intervention for hepatic metastases, US still showed liver metastases. US showed a tumor with regular contour, hyperechoic, homogeneous, with a diameter of 12 mm, in contact with the left suprahepatic vein.

Computed tomography (CT) could not be performed in one patient because she exceeded allowable weight, so of the 20 patients undergoing this investigation, one result was inconclusive, and from the remaining 19, 4 results were positive, with tumor size over 1 cm.

The sensitivity of CT was unsatisfactory: 4 positive results out of 19 (21.05%). CT failed to detect liver metastasis, but identified lymph node metastasis in one patient.

Magnetic resonance imaging (MRI) was performed in 18 patients and was diagnostic in 11 of them, recording a detection sensitivity of 61.11%, including infracentimetric tumor size.

Figure 1. Sensitivity of imaging methods (%)



Endoscopic ultrasound (EUS) was performed in all patients, being able to identify formations in 17 out of 21, showing a diagnostic sensitivity of 81%.

In two patients, the results were inconclusive, as inhomogenous, poorly delineated areas were seen in the head and tail of the pancreas, but without clear identification of a tumor.

Excluding the other two patients in whom EUS failure was caused by lack of cooperation or poor conditions related to patient preparation, reporting the results of the remaining 19 patients who underwent EUS, we can say that its sensitivity was 89.47%.

Regarding EUS location of insulinomas, in our study, 44.44% were proximal (head and uncinate process), 27.77% in the body and the same percentage in the tail. The mean size of insulinomas was 1.958 cm (range 0.91 cm - 5.37cm). Most of them were between 1 and 3 cm (13 out of 18, meaning 72.2%).

Preoperative diagnosis and treatment

The preoperative topographic diagnosis was possible in 18 (86%) of the 21 patients with insulinoma. It was not possible in 3 patients, in whom all localization imaging investigations were negative, but in the presence of biological and symptomatic confirmed insulinoma.

In these patients surgical intervention was not performed, the solution consisting of reevaluation, follow-up and palliative treatment.

17 of 21 patients with insulinoma were referred to surgery. In addition to the 3 patients where the tumor could not be located, another patient with rare symptoms, drug controlled, was not operated because he did not accept surgery; he is being monitored clinically and by imaging methods.

At surgical exploration, the abdomen is initially investigated for evidence of metastatic disease. The pancreas is then completely exposed, allowing palpation of the entire pancreas.

Intraoperative ultrasonography and palpation can be performed at this time in order to effectively localize and guide in the dissection of the tumor. Since most insulinomas are benign and solitary, tumor enucleation is the procedure of choice, when is technically feasible (3).

In patients undergoing surgery, intraoperative diagnosis was made by intraoperative palpation of the tumor and/or intraoperative ultrasound. In 8 patients the intraoperative diagnosis was established by manual palpation of the pancreas and in 8 patients the two techniques were combined (pancreas palpation and intraoperative ultrasound).

In our cases, intraoperative ultrasound combined with a manual exploration of the pancreas showed a

diagnostic sensitivity of 94.11%. The tumor could not be identified at the first surgery on a patient, but intraoperative ultrasound managed to locate it at the second intervention.

Table 3. Imaging methods and EUS location of insulinomas

	Patient	Gender	Age	US	CT	MRI	EUS tumor localization within the pancreas
1	PA	F	43	negative	negative	negative	between the body and the tail, near the posterior part of the gland
2	CC	F	37	negative	negative	negative	neck
3	ATI	F	26	negative	patient's weight was above the upper limits accepted for CT	patient's weight was above the upper limits accepted for MRI	located in the tail, posterior, near the left kidney
4	ME	F	46	positive, after EUS	negative	positive, after EUS	near the confluence, posterior
5	TC	M	53	negative	negative	negative	head
6	BM	M	41	negative	negative	positive, after EUS	head (uncinate)
7	AA	F	27	negative	negative	negative	fare tail, posterior, between spleen, left kidney and adrenal gland
8	SG	M	75	liver metastatis	negative	positive	head area
9	AZA	F	52	negative	negative	positive	border between body and tail, posterior
10	RL	F	50	negative	positive	positive	inhomogeneous head; cystic tumor in the tail
11	MNC	F	30	negative	positive	positive, after EUS	neck, posterior, near the confluence
12	SM	F	61	negative	negative	positive	neck
13	MI	M	72	not performed	negative	positive	body
14	LM	F	63	negative	negative	negative	tumor not located
15	GC	M	45	negative	negative	not performed	inhomogeneous head
16	VM	F	52	negative	negative	negative	head (uncinate), posterior
17	AD	F	38	negative	inconclusive	not performed	inhomogeneous tail
18	UV	M	43	negative	positive	negative	head (uncinate)
19	ELE	F	36	positive	negative	positive	tail
20	CG	F	38	negative	positive	positive	tumor not located
21	GC	F	43	negative	negative	positive, after EUS	between body and tail

Grade and differentiation

According to European Neuroendocrine Tumors Society (ENETS), detailed description of macroscopic, microscopic and immunohistochemical features is absolutely necessary to confirm the diagnosis of NTP and allow correct classification. The grade of a tumor refers to its biologic aggressiveness. For NTP, the grading system is based on the rate of proliferation, which is defined by the number of mitoses per 10 high-power microscopic fields or per 2 mm^2 (mitotic rate), or as the percentage of tumor cells that immunolabel positively for the Ki-67 antigen (Ki-67 index). An important role in insulinoma histological diagnosis is played by immunohistochemical analysis. NTP can also be classified based on differentiation, which refers to the extent to which cancerous, or neoplastic, cells resemble normal cells. Well differentiated NET cells produce large amounts of secretory granules with diffuse immunoexpression of

neuroendocrine markers. In contrast, poorly differentiated NTP have atypical, sheet-like, diffuse and irregular nuclei, less cytoplasmic secretory granules, and limited biomarker immunoexpression. General markers of well-differentiated NET include cytosolic markers (neuron-specific enolase and PGP-9.5), markers associated with small vesicles (synaptophysin and synaptotagmin), markers associated with secretory granules (chromogranin A and HISL-19). Specific cell markers include insulin and proinsulin hormones. Poorly differentiated tumors do not express cell markers but can be positive for some of general markers. Endocrine differentiation may be confirmed at immunohistochemical analysis with neuroendocrine markers such as chromogranin A or synaptophysin. In well-differentiated NTP, determination of malignancy is straightforward only if the tumor invades adjacent organs or a distant metastasis is present. Histologic confirmation of complete excision and the benign nature of the insulinoma are essential (7).

Histological findings were available for our study in 7 of the 17 operated patients (tests were not available for the patients that were included in the first years of the study and we did not manage to recover the paraffin blocks to make them new tests). In 6 of these, the neuroendocrine tumor was identified; in one patient the tumor was not found and the absence of exact location of reccurent insulinoma led to a palliative treatment.

Table 4. Surgical interventions performed in patients from the study

	Patient	Previous surgery for insulinoma	Surgical intervention	Type of intervention
1	PA	no	yes	Surgical enucleating
2	CC	yes	yes	Surgical enucleating
3	ATI	no	yes	Surgical enucleating
4	ME	no	yes	Surgical enucleating
5	TC	yes	Patient refused surgical intervention	Patient refused surgical intervention
6	BM	no	yes	Tumor resection
7	AA	no	yes	Tumor resection
8	SG	yes	yes	Follow up (liver metastasis)
9	AZA	no	yes	Tumor resection
10	RL	no	yes	Tumor resection
11	MNC	no	yes	Duodenopancreatectomy
12	SM	no	yes	Laparoscopic enucleation
13	MI	no	yes	Tumor resection
14	LM	no	no	Follow up
15	GC	no	no	Follow up
16	VM	no	yes	Surgical enucleating
17	AD	no	no	Follow up
18	UV	no	yes	Tumor resection
19	ELE	no	yes	Tumor resection
20	CG	yes	yes	Tumor resection
21	GC	yes	yes	Surgical enucleating

Dietary treatment and medication were recommended especially for preoperative preparation of patients, aiming to reduce the frequency and severity of hypoglycemic symptoms and consisted of administration of frequent meals rich in carbohydrates and possibly an intravenous glucose solution and Verapamil.

In patients in whom the tumor could not be located with any imaging technique and for the patient who refused the surgery because of light and rare symptoms, and last but not least, in patients whose surgery was not curative, treatment was palliative. Drugs used for this purpose were: Verapamil tablets 80 mg or 40 mg 3 times daily, Octreotide (Sandostatin 0.1 mg every 8 hours subcutaneously) and 1 mg Glucagon kit (glucagon) intramuscular injection (the thigh or deltoid) in case of impaired consciousness (the latter was used in one case). These were indicated by the severity of symptoms. General instructions were: diet, frequent small meals rich in carbohydrates, avoidance of high physical exertion, and also periodic medical examination. Their combination succeeded to control the clinical manifestations in patients in whom surgery was not possible as a solution.

Surgical treatment was applied to 17 of 21 patients with insulinoma. In all 17 patients the tumor was localized by EUS. Some patients had two or even three surgical interventions. In 4 of them, EUS was performed before the second intervention, in another EUS was diagnostic and performed several times, but it did not provide an exact location of reccurent insulinoma. One patient underwent two surgeries, the first following suspicion of insulinoma, so partial pancreatic resection was performed, but no evidence of neuroendocrine tumor was found in the excised pancreatic tissue (blind partial pancreatic resection did not include tumor). Operative failures were due to lack of precise localization of the tumor and presence of metastases at EUS (inhomogeneous head/tail), but there were only 3 such cases. Regarding the 4 patients with negative EUS results, 3 of them were not operated because of failure to locate the tumor, therefore they are followed up. The fourth patient underwent surgery (tumor resection) with a good subsequent recovery. Among patients with positive EUS diagnosis surgery was successful, intraoperative diagnosis being made by intraoperative palpation of the tumor and/or intraoperative ultrasound showing the same location of the tumor as in EUS. There was one exception in a patient with metastasis that was followed up.

Recurrence of symptoms has led, ultimately, to a secondary surgical intervention (resection of pancreatic head and duodenum); this time intraoperative ultrasound confirmed location of the tumor and its excision was performed successfully. It is important to note that EUS was not performed in this patient. Another case is of a patient who underwent corporeo-caudal pancreatectomy without remission of hypoglycemic syndrome even if the tumor was localized preoperatively by EUS. Three years later EUS revealed tumor recurrence in the remaining parenchyma, which was enucleated with good recovery. Of the 7 enucleations, 3 (43%) were for tumors located in the pancreatic head.

Complications

The immediate postoperative complications occurred in 8 of the 17 patients who had surgical intervention, so we can say that postoperative morbidity was 47.05%. The complications are presented in table 5.

Table 5. Postoperative complications

Complication	Number
Transient hyperglycemia	4
Late postoperative pancreatic pseudocyst	2
Postoperative anemic syndrome	1
Transient pancreatitis	1
Postoperative eventration	1
Permanent diabetes mellitus	1
Exocrine pancreatic insufficiency	1
Malabsorption	1

The most common complication was transient hyperglycemia that occurred in 4 patients (23.52%), ranging between 3 and 15 days, with values between 130-170 mg/dl.

Diabetes mellitus occurred in a patient who underwent cephalic duodeno-pancreatectomy and exocrine pancreatic insufficiency in a patient who

underwent a corporeo-caudal pancreatectomy followed by enucleation after three years.

Postoperative eventration and pancreatic pseudocysts were resolved surgically.

Metastases and recurrence

Malignancy is suggested by large tumor size, presence of metastases or vascular invasion. Tumors larger than 3 cm raise the suspicion of a malignant insulinoma. Other biological explorations advocating for malignancy are: high insulinemia, proinsulin large proportion (90%), elevated C-peptide. Ki-67 index is also a predictive factor for malignancy. Necrosis and high mitotic rate also correlate with malignancy. (5, 6, 7)

Concerning metastasis, ultrasound identified a hyperechoic area, with regular contour, on hepatic segment IV. This patient was previously operated for insulinoma with liver metastases: corporeo-caudal pancreatectomy and splenectomy were performed and liver metastases were resected in segments V, VI, VII of the liver. The therapeutic approach involves close patient monitoring and guidance for oncology service for cancer therapy. Liver metastases were demonstrated in 2 more patients by MRI. MRI confirmed the one found at US too. 5 patients had postoperative recurrences at varying intervals of time. In one patient in whom symptoms reappeared after seven years, imaging methods could not identify the tumor so he received palliative treatment (Verapamil 240 mg per day orally, Sandostatin 0.1 mg every 8 hours subcutaneously, diet, avoiding intense exercise). In another case, 3 years after surgery, at the EUS reevaluation a tumor was observed suggesting a possible recurrence; the patient refused surgical intervention. Excluding those 2 patients with liver metastases and the 5 with recurrence, we can say that the remaining 10 received curative surgery. And out of the 21 patients with insulinoma in the study, the remaining 4 underwent a palliative treatment, with periodic evaluation to locate the tumor. Where recurrent tumor could be identified, but the patient refused surgical intervention, anatomical location was the same as the primary tumor.

Evolution and mortality

Postoperative evolution was generally favorable, with 0% mortality. Curative surgery was followed by the disappearance of hypoglycemic symptoms, good clinical condition and weight loss.

DISCUSSION

Patients with insulinoma have symptoms of excessive insulin release with consecutive hypoglycemia, namely neuroglicopenic and adrenergic symptoms. The delay in establishing the diagnosis is explained by misleading and nonspecific clinical presentation (5, 7, 8). A high index of clinical suspicion is therefore essential. Eight of our patients were hospitalized in the neurology and psychiatry clinics and mistreated. Of them, four patients were diagnosed with epilepsy and received anticonvulsant treatment, and the other four patients were labeled with neurological disorders, anxiety disorders or thyroid dysfunction.

Between the onset of symptoms and diagnosis days, months, years or even decades can pass. This situation is found in our patients, the correct diagnosis being established over a period of symptomatic evolution averaged 25.14 months, but there are delays that have reached 10 years.

Diagnosis is based on Whipple's triad. The biochemical diagnosis of insulinoma is supported by the presence of hypoglycemia and inappropriately elevated insulin levels during prolonged fasting. In most patients (90%), insulinomas appear as single pancreatic tumors. In our study all patients were classified in this category – single pancreatic tumor – and the most common location was the proximal one (head and uncinate process) - 44.44%.

Most insulin-secreting tumors are small, so they are a diagnostic challenge for physicians, endocrinologists, gastroenterologists, surgeons and radiologists. In our case, most of them were between 1 and 3 cm (average size 1.9 cm). The role of imaging is to detect and provide precise information on anatomical location and staging before surgery.

Ultrasound, CT and MRI showed poor sensitivity. In our case it was 10% for US (literature 9-79.3% (6,

10)), positive results being obtained in patients with tumor located proximally (pancreatic region most accessible for abdominal US exploration), at the junction head – pancreatic body, and in only one tumor located caudally, but large (63/43 mm).

The diagnostic utility of somatostatin receptor scintigraphy is limited by the fact that only about 40-50% of them have somatostatin receptors, so that a negative scan cannot exclude the presence of an insulinoma. Octreoscan was performed in two patients; in one was not conclusive, in the other one revealed metastatis.

Studies show that PET (Positron Emission Tomography) is a sensitive imaging method to locate insulinoma with higher sensitivity than CT or MRI (26). PET was performed in three patients in our study, identifying metastatic insulinoma in only one of them. It should be noted that both Octreoscan and PET-CT became available later; however none of them have helped us greatly in clarifying the diagnosis.

EUS is particularly useful. EUS detection rates vary in different studies from 40% to 93% (6). EUS located the tumor in a patient where US and CT were negative. The typical lesion at EUS is round, smooth, hypoechoic, located mostly in the pancreas. Neuroendocrine tumors of the pancreas are usually slow growing, indolent, and growth is still a major determinant of survival in these patients. Because they usually grow slowly, their metastatic potential is relatively low and there are specific criteria to predict their behavior (the distinction between benign and malignant tumors is based on the presence of metastasis), so long-term monitoring is necessary. Currently, the most important prognostic factor is the presence or absence of metastases. Even metastatic tumors grow slowly, the prognosis in these patients being relatively good compared with non-endocrine pancreatic neoplasms (27, 28).

10% (5.8-15%) of insulinomas are malignant and already present liver and locoregional lymph node metastases at diagnosis. In our study three patients with insulinoma had liver metastases (14.28%).

Treatment options for pancreatic neuroendocrine tumors require a multidisciplinary approach and depend on the presence or absence of metastases. Surgical resection is the only potentially curative approach. (29)

The main technical options available are: enucleation and pancreatic resections, the choice of either method being determined by location, size and number of tumors. Enucleation is generally preferred because it strictly removes the lesion without unnecessary sacrifice of healthy pancreatic tissue; in our study was performed in 7 of the 19 surgeries (30).

Intraoperative diagnosis was made by intraoperative palpation of tumor and/or intraoperative ultrasound. Intraoperative ultrasonography is valuable for locating small lesions and has an overall sensitivity of 80-100% (5), being more sensitive than all other preoperative imaging techniques and better than physical examination performed intraoperatively by the surgeon. In our case, intraoperative ultrasound combined with a manual exploration of the pancreas showed a diagnostic sensitivity of 94.11%.

Postoperative complications – transient hyperglycemia, anemia, transient pancreatitis, pancreatic pseudocyst, diabetes mellitus, exocrine pancreatic insufficiency, malabsorption, eventration – occurred in 8 of 17 patients (postoperative morbidity 47.05%).

In one case, the tumor was not found by intraoperative palpation, probably due to its deep location, but was easily detected by intraoperative ultrasound evaluation.

In metastatic disease the most important therapeutic goal is to reduce the life-threatening hypoglycemic syndrome. This can be achieved by surgical removal of metastases or a variety of other cytoreductive techniques, radiofrequency ablation, with good results. An active approach to patients with advanced disease may result in longer survival and prevention of hypoglycemia.

Medical treatment was used to prepare the surgical one aiming at reducing the frequency and severity of hypoglycemic symptoms in patients whose tumor could not be located with any imaging techniques and in the patient who refused surgery because of mild

and rare symptoms and, not least, in patients whose surgery was not curative (palliative treatment).

Drugs used for this purpose were: Verapamil, Octreotide (Sandostatin) and Glucagon (glucagon). They were indicated by the severity of symptoms. General instructions were: diet, frequent small meals rich in carbohydrates, avoidance of high physical exertion, and also periodic medical examination. Their combination succeeded to control the clinical manifestations in patients in whom curative surgery was not possible as a solution (31).

Evolution was generally good, with 0% mortality, and curative interventions were followed by the disappearance of hypoglycemic symptoms, good clinical condition and weight loss. Unfortunately, there were some failures of surgery, especially when the location of the tumor has not exactly been made preoperatively or intraoperatively, more likely associated with metastatic disease.

CONCLUSIONS

The challenging aspect of treating insulinomas is localizing the tumors. Diagnosis has three steps: identifying the clinical syndrome, biological diagnosis with hormonal determination and topographic diagnosis. Due to nonspecific symptoms, a high index of suspicion is required. The supervised fast is the classic diagnostic test for insulinoma. Histopathological diagnosis is essential for diagnosis and therapeutic management. Native CT or CT with intravenous iodinated contrast material had low sensitivity in detecting insulinomas and liver metastases, but identified in our study, lymph node metastases. MRI is more sensitive than CT and can diagnose insulinomas with infracentimetric size; MRI is also a technique of choice for highlighting possible metastases in the liver. EUS can locate the tumors and describes the size, shape, contour, echostructure and relationship with adjacent structures. High resolution of EUS allows detection of lesions with very small diameter, is safe and minimally invasive. EUS is the imaging investigation of choice in preoperative diagnosis of insulinomas. Surgical resection remains the mainstay of treatment for patients with localized disease. Future studies must lead to further improving therapeutic options for patients with this disease.

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Article received on March 7, 2015 and accepted for publishing on April 19 2015.

Endoscopic faces of Helicobacter Pylori infection

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Abstract: *Introduction: The infection caused by H. pylori appears secondary after a bacterial colonization of the stomach and the initial portion of the small bowel. H. pylori –infected patients can develop gastritis, peptic ulcer, stomach cancer or MALT lymphoma. H. pylori infection is defined by WHO like a type I carcinogen, its role in gastric carcinogenesis being supported by the greatest researchers.*
Objectives: In this study our purpose was to determine the endoscopic appearances in H. pylori infection quoted in medical literature until now and the frequency of their appearance in our group of interest.
Materials and methods: In this study it was made an analytic study in which it was realized a retrospective cohort investigation at the Emergency Central Military and University Hospital "Dr. Carol Davila" Bucharest, gastroenterology branch –endoscopic department between 18.12.2012-21.08.2013 on 1694 patients between 18 and 92 years old, with the medium age of 55 years old.
As a diagnostic method for H. pylori infection we used superior digestive endoscopy during which were taken biopsies and it was made a fast urease test.
Results: Regarding the variation of the endoscopic aspects at the population of study, we have found gastritis with all its aspects (which was Sidney classified) in the biggest percentage meaning 59.3% of the cases, followed with a percentage of 18.8% by those without any endoscopic abnormality, and then in 10,33% of the cases we have found peptic ulcer. With a smaller percentage, under 10%, we have found duodenitis at 8.67% of this patients, and finally the most severe lesions represented by gastric cancer and lymphoma were found at 2,7% of the H.pylori infected patients.

INTRODUCTION

H. pylori is the most common infection found in humans, is a transmissible chronic infectious disease in which clinical manifestations are preceded by a long asymptomatic period. Helicobacter pylori is a gram-negative bacterium, the infection occurs due to bacterial colonization of the stomach and the initial portion of the small intestine and it is found on the luminal surface of the gastric epithelium. Was first isolated by Warren and Marshall in 1983.(1) In the entire world more than 50 % of the population have the infection into their upper gastrointestinal tract, the infection is found more often in the developing countries and with a significantly low percent in industrialized countries (2).

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The transmission routes in developing countries are largely represented by water, while in industrialized countries the transmission routes are directly, and the persons are infected from saliva, vomitus and others fluids.(3)(4)

Helicobacter pylori infection leads to development of gastrointestinal pathologies such as duodenal or gastric ulcers, many forms of gastritis, gastric cancers, and gastric mucosa-associated lymphoid-tissue (MALT) lymphoma. At the patients infected with Helicobacter pylori the risk of developing one of this pathologies varies widely in population, but a lot of the patients will not have any of this complications. (5)

By altering the normal gastric secretion, the bacterium produces local injury of the gastric mucosa especially at the antrum and distal part of the stomach. The most common pathology is gastritis and ulcers. At patients with adenocarcinoma, a known complication of Helicobacter pylori, the patients suffer a pangastritis with highly secretion of the body and antrum of stomach.(6)

The standard treatment for Helicobacter pylori includes PPI and three antibiotics which involves taking for 7 to 14 days and no single drug cures the infection. The proton pump inhibitors are decreasing the production of acid from the stomach and which allows the tissues to heal. Beside PPI also are recommended two antibiotics such as clarithromycin (500 mg, bid), amoxicillin (1 g, bid) and metronidazole. (7)

Unfortunately several clinical studies have shown that the triple therapy scheme from Europe the success rates are only 20-30% (10)(11). And therefore the detection of this bacteria can be realized in early stages and we can prevent various complications determined by it (especially gastric cancer) and use the treatment guides which were very well established within years has declined to the limit of 80% or less (8)(9) and in some countries

In this study our purpose was to determine the types of endoscopic aspects found in H. pylori infection quoted by now in medical literature and the frequency of their appearance in the group of study.

Materials and method

This study was realized between 18.12.2012-21.08.2013 on 1694 patients between 18 and 92 years old, with the medium age of 55 years old.

Inclusion criteria of endoscopic examined patients were represented by: patients with age over 18 years, unspecific digestive symptomatology to whom can associate alarm signals, the endoscopy indication made by the doctor, presence of a suggestive symptomatology for gastric or duodenal pathology, pain in the upper abdominal region or retrosternal, postprandial discomfort, distended bowel, early satiety, abdominal discomfort, pirosis, nausea, vomiting, anorexia, weight loss.

Exclusion criteria for this patients were similar with those for superior digestive endoscopy and are represented by: patients under 18 years , shock, acute myocardial infarction, peritonitis, acute perforation, fulminate colitis, recent colonic surgery, patient's refuse, low cooperation of the patient, coma (except intubated patient), arrhythmias, acute myocardial ischemia.

In this study were included 1964 adults with age between 18-92 years old, as it is shown in table 1.

Table 1. Gender distribution of patients

Male	Female
866	828

As a diagnostic method of H. pylori infected patients, we used superior digestive endoscopy. Within the intervention were taken biopsies and were realized fast urease tests.

Fast urease test is based on the principle that excessive urease produced by H. pylori hydrolyses urea in ammonia. Therefore we have an increase of the pH of the environment in which we have introduced the bioptic fragment, determined with a colour indicator.

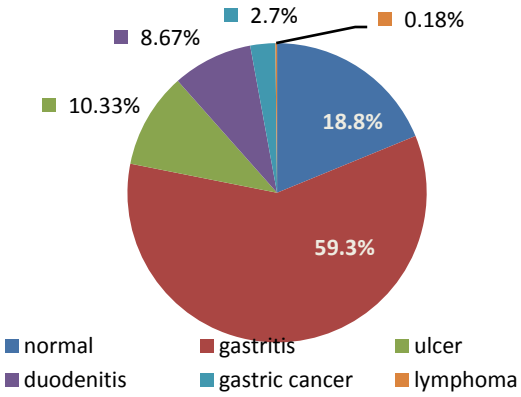
Although this test has a high specificity and sensibility, in general over 90%, the result seems to be influenced by certain drugs such as: PPI (proton pump inhibitors), antibiotics, H2 receptor antagonists and bismuth.

According to the large amount of data given by the medical literature, H. pylori infection produces various gastric and duodenal lesions with a low to high severity, and here we can mention gastritis, duodenitis, gastric and duodenal ulcer, gastric cancer and lymphoma.

In the group of patients with indication for superior digestive endoscopy, we have managed to visualize the endoscopic aspect mentioned up above, with a small mention that some of this patients had a normal endoscopic aspect.

Regarding those varied endoscopic aspects found in our group, we have determined that gastritis with all its aspects according to Sidney classification (described below) has the highest percentage of 59.3%, followed by normal endoscopic aspect with 18.8%, then we found ulcer in 10.33% of cases, duodenitis 8.67%, and finally the most severe- gastric cancer and lymphoma – only 2.7%, respectively 0.18% of the total examined group.

Figure 1. Distribution of endoscopic aspects found at endoscopic examined patients



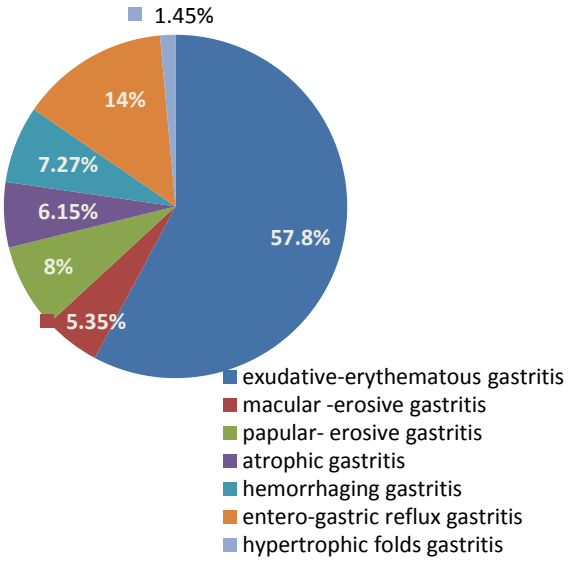
According to Sidney classification we determined the following types of gastritis: exudative-erythematous gastritis, macular-erosive gastritis, papular-erosive gastritis, atrophic gastritis, haemorrhaging gastritis, entero-gastric reflux gastritis and hypertrophic folds gastritis.

In all the endoscopic examined patients, from the total percentage of 59.3% that was represented by gastritis we have determined that the most frequent is exudative-erythematous gastritis, which represents better than a half with 57.8%.

The following as a frequency is represented by entero-gastric reflux gastritis with a percentage of 14% which was followed by papular-erosive gastritis, haemorrhaging gastritis, atrophic gastritis, and macular-erosive gastritis, with small differences between them, these representing 8%, 7, 27%, 6, 15%, respectively 5.35%.

Finally the less frequent was hypertrophic folds gastritis with a percentage of 14.45%.

Figure 2. Gastritis distribution according to Sidney classification of endoscopic examined patients



In the following figure we have presented gastritis distribution according to Sidney classification at the H. pylori-infected patients, and we determined that from all the cases with gastritis the most frequent was exudative-erythematous gastritis with a share of 61%. Must be mentioned here that 21.43% from all the cases with gastritis were infected with H.pylori.

The following places as a frequency, but still at pretty big difference from the first mentioned, were occupied by papular-erosive gastritis with 11.8%, macular-erosive gastritis with 10.62%, and entero-gastric with 6.7%. We can mention here that the percentage of these three determinations of gastritis are at the opposite pole from their situation in general examined population, where entero-gastric reflux gastritis has a bigger percentage than the

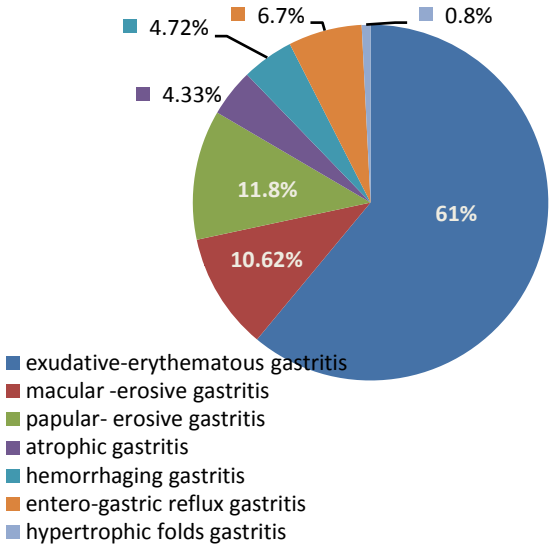
others two. Now if we make a parallel between the occurrence of those gastritis types and the rate of occurrence in general population we can tell that 40.3% from the total macular-erosive gastritis cases and 30% from all the total popular-erosive gastritis found in general population appear in H. pylori infection, in contrast to entero-gastric reflux gastritis where only 9.7% from the cases occur in H. pylori infection.

Regarding haemorrhaging gastritis and atrophic gastritis, these have a smaller frequency of occurrence within the pale of infected patients, with a percentage of 4.72%, respectively 4.33%.

If we relate again at their distribution in general population we can observe that 14.3% from the total number of cases with atrophic gastritis is found in H. pylori infection. Haemorrhaging gastritis occurs in 13.2% of all the total haemorrhaging gastritis found in general population.

The smallest value as a frequency of occurrence in the H. pylori infected patients belongs to gastritis with hypertrophic folds with a percentage of 0.8% and we mention here that this value represents 11.11% from all the total cases of hypertrophic folds gastritis found at general population that was endoscopic examined.

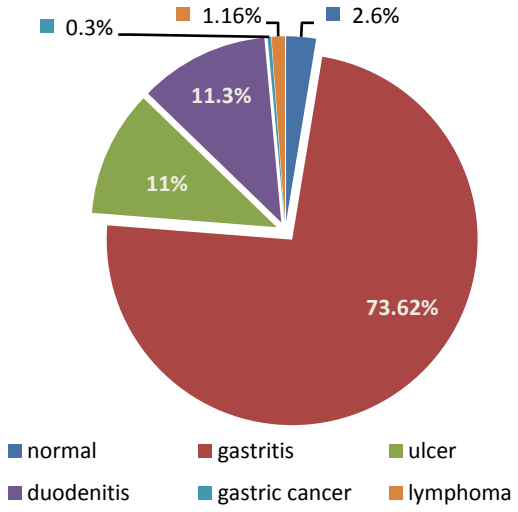
Figure 3. Gastritis distribution according to Sidney classification of positive fast urease test patients.



We observed at the persons in which was determined a positive fast urease test that the most frequent determination of H. pylori infection is represented by gastritis, which was found in 73.62% of the cases, a percentage up high as against general population where gastritis was determined in 59.3% of the cases. If we make a parallel of gastritis cases found at H. pylori infected patients and the cases found in general population we can observe that 20.3% of the gastritis cases occurred secondary to H. pylori infection.

The following two places as a frequency are occupied by ulcers and duodenitis with a small difference between them of only 0.3%, these two having the percentage of 11%, respectively 11.3%.

Figure 4. Distribution of endoscopic aspects found at patients with positive fast urease test.



It is known that H. pylori infection can determine ulcerated lesions with gastric and duodenal localization.

From all the ulcerated lesions that were endoscopic discovered at the general examined population we determined that is a significant difference between these two types of localization, the one with the biggest percentage being the duodenal ulcer meaning 64.2%, in comparison with gastric ulcer which represents 35.8%.

In figure 6 was determined the distribution of ulcerated lesions at male gender with H. pylori

infection where we can observe that three quarters from all the ulcer cases found at this group belongs to duodenal ulcer and the rest of 25% belongs to gastric ulcer.

Figure 5. Distribution of ulcer types according to its localization at endoscopic examined patients.

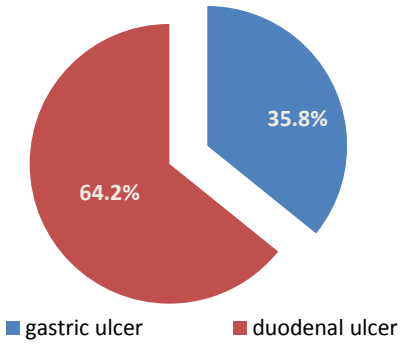
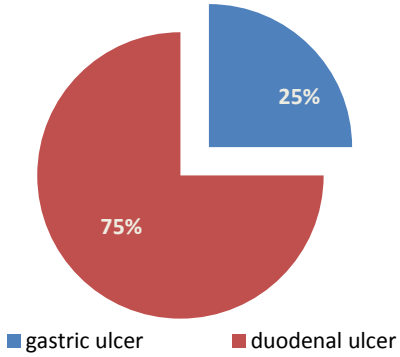


Figure 6. Distribution of ulcer types according to its localization at male gender patients with positive urease test



CONCLUSIONS

1. Helicobacter pylori was found in 52.22% of the cases tested for this infection and in 15.23% of the general population with superior digestive symptomatology.
2. Helicobacter pylori infection doesn't have gender predominance, being equivalent at both sexes.
3. In 97, 4% from Helicobacter pylori infected cases was determined a pathologic endoscopic aspect, the most frequent being gastritis with all its forms.
4. The most frequent gastritis type met in Helicobacter pylori infection was exudative-erythematous gastritis.
5. From all the ulcerated lesions met in Helicobacter pylori infection, the most frequent we have determined was at duodenal level.
6. Almost 20% from all the gastritis cases, ulcer and duodenitis were found in Helicobacter pylori infection.
7. All the lymphoma cases were found at Helicobacter pylori infected patients.

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Article received on May 30, 2015 and accepted for publishing on June 15 2015.

Multidisciplinary approach to anemia

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Abstract: *Introduction: We present the case of a 65 years- old woman who was admitted with a severe macrocytic anemia Hb= 5.7g/dl and diffuse bone pain. Biologically she has moderate thrombocytopenia 35 000/µl, a hepatic cytolysis and cholestatic syndrome.*

Material and method: The patient was extensively evaluated before presentation for a mild iron - deficiency anemia for which she underwent endoscopic examination of the upper and lower gastrointestinal tract- normal. The bone marrow aspiration on admission revealed a marked hyperplasia of the erythroblastic line with ~50% basophilic erythroblasts suggesting a regenerative erythroid hyperplasia. These changes along with the marked reticulocytosis on the peripheral blood smear oriented us towards a hemolytic anemia; Folic acid, vitamin B12, autoimmune tests and hemolytic tests were all normal. We continued the investigations with a thoraco-abdomino-pelvic computed tomography which identified diffuse demineralization, vertebral compactation and pelvic stress fractures. The breast examination revealed a right breast nodule, but the breast ultrasonography pleaded for benignity. Lacking a clear definitive diagnosis we decided to perform a bone marrow biopsy.

Results: The osteo- medullary biopsy pointed towards a medullar invasion from a lobular mammary carcinoma; In these circumstances we performed an ultrasound guided biopsy of the right mammary lump thus histologically confirming a tumoral invasion of the bone marrow with subsequent anemia. The patient started chemotherapy in the Oncology ward.

Conclusion: The particularity of this case consists in the pattern of anemia, which initially seemed iron deficient and afterwards macrocytic – apparently hemolytic and was actually due to the tumoral medullar invasion and also the nonspecific ultrasonographic appearance of the breast tumor.

Keywords: *anemia, breast cancer, Evans syndrome*

INTRODUCTION

Anemia is a clinical feature in many emergency admissions to hospital. It is associated with increased mortality, morbidity and length of hospital stay in patients presenting with a range of conditions. If discovered in primary care it is seldom incompletely investigated. Although the most frequent cause of anemia is blood loss, as clinicians, we often find ourselves in front of complex and intricate causes which lead to a severe deficit. Therefore the management of such patients remains problematic. The prevalence of anemia under widely different conditions and the fact that anemia is managed by clinicians with a broad variety of backgrounds have fostered the need of multidisciplinary teams of experts (primary care physician, gastroenterologist,

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hematologist) to manage such cases.

The most important aspect of treating anemia lies not in correcting it and discharging the patient when stabile, but in identifying the cause and reversing it in order to prevent recurrences.

CASE PRESENTATION

Disease history

We present the case of a female patient aged 65, living in the country area, whose history began in January 2015. She consulted her family physician for fatigability and after having a series of blood test taken was prescribed iron substitution for 4 months. She had microcytic hypochromic anemia – her hemoglobin was 9.1g/dl, MCV = 72.5 fL, MCH = 24.3 pg and low blood level of iron = 39 ug/dl. Her family physician also recommended some tumor markers (aFP, CEA, CA 19.9) which turned out to be negative.

The patient thoroughly followed the treatment, but at the end of the treatment, when she performed a control full blood count her hemoglobin level was lower. This is the moment when she was referred to us.

Gastroenterology admission

On admission she was pale, stable hemo- dynamically BP = 120/70 mmHg and HR = 90 bpm and alert, asthenic . She reported not loosing any blood and on rectal examination there were no signs of fresh blood or melena.

Her laboratory data showed anemia with hemoglobin 7.1 g/dl, MCV 110 fl, MCH 35.6pg, blood iron 155 micrograms/dl, platelets 39.000/µl. So we are now facing a macrocytic anemia with a normal iron level and associated thrombocytopenia. Peripheral smear shows macrocytosis, numerous polycromatophylic red cells agglutination, erythroblasts 8% and 7.9% reticulocytes, in conclusion hyperplasic marrow with pronounced erythropoiesis – regenerative erythroid hyperplasia.

Endoscopic examination were performed: upper endoscopy showed duodeno-gastric reflux and small diaphragmatic hernia and colonoscopy revealed hemmorhoidal disease with absent signs of bleeding.

After excluding a bleeding site and because the anemic syndrome was now macrocytic protein electrophoresis, folic acid and vitamin B12 levels were also performed and both were normal.

Because the patient was feeling worse and the hemoglobin was dropping despite blood transfusion and iron supplementation she was transferred to Hematology for further investigation.

Hematology admission

In addition to fatigability, after 4 days in the gastroenterology ward, the patient now reports bone pain, without loss of range of motion. The pain responds to usual analgesics. On examination she is stable and on physical examination we discover superficial bilateral axillary lymph nodes 1.5 cm diameter, mobile and non tender and a right mammary nodule on breast examination .

Laboratory test now shows a worsening anemia and thrombocytopenia with a hemoglobin of 5.7 g/dl, MCV 114 fl, platelets 35.000/µl, Iron 304 micrograms/dl and hepatocytolytic and cholestatic syndrome: AST 108 UI/l, ALT 45 UI/l, GGT 228 UI/l (N 7-50), alkaline phosphatase 180 UI/l (N 38-91 UI/l), LDH 1081 (N 266-500). Hepatic viral serology was negative for hepatic viruses B and C. Coombs test and cold agglutinins test were also negative.

Ultrasonography was also performed and showed fatty liver and normal sized spleen without abdominal adenopathy, the heart was normal. Soft tissue ultrasonography shows bilateral axillary lymph nodes with apossible non-inflammatory character and 1.5 cm diameter. We also identified smaller lymphadenopathy bilaterally in the cervical area, resembling the appearance. On breast examination we described fibromatous placard in the supero-external area of the right breast with the long axis parallel with the skin, 4a BIRADS classification.

The biological panel strongly resembled Evans syndrome on admission, so we immediately decided to start treatment with high doses of dexamethasone while continuing investigations.

The non-inflammatory character of the axillary adenopathies prompts searching for additional lymph

nodes. Therefore tomography of the thorax and abdomen was performed and it identified diffuse demineralization of the scanned bones, numerous vertebral compression fractures and stress fractures of both iliac wings, during consolidation.

Figure 1. Right axillary lymph node, 1.48 cm diameter

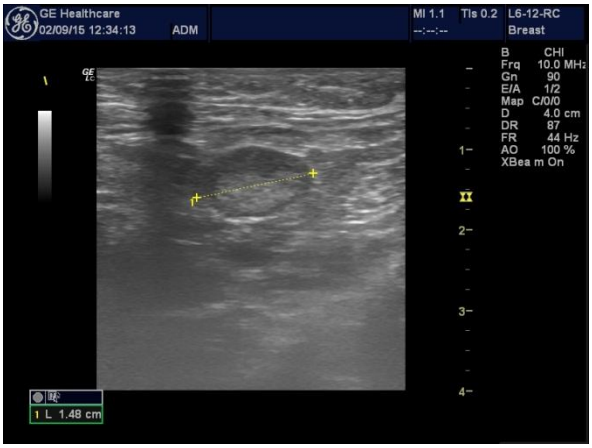
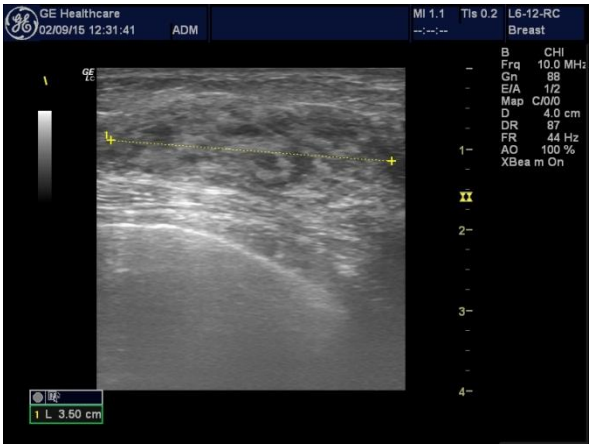


Figure 2. Right breast nodule, 3.5 cm diameter



Not having a definite diagnosis and because our patient was feeling worse and her hemoglobin was stationary despite treatment, we performed a diagnostic test.

A bone marrow biopsy was performed. Histologically and imuno-histochemically a metastatic lobular carcinoma of the breast was identified. Faced with a metastasis from a still not identified tumor, we returned to the fibromatous placard we saw in the right breast and an echo-guided biopsy of the nodule was performed. The biopsy revealed a lobular carcinoma strongly positive for estrogen receptors (ER) and negative for progesterone receptors (PR) and E-cadherin by immunohistochemical staining. The tumor marker was intensely elevated – CA 15-3 530 mg/dl.

Figure 3. Thoraco-abdominal CT showing diffuse demineralization and vertebral compression fracture

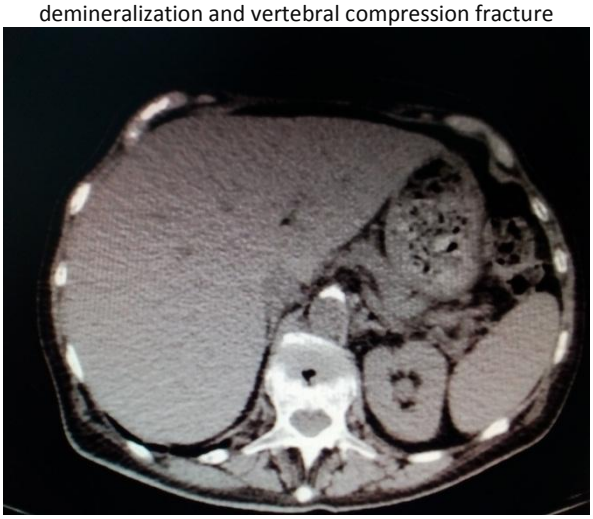
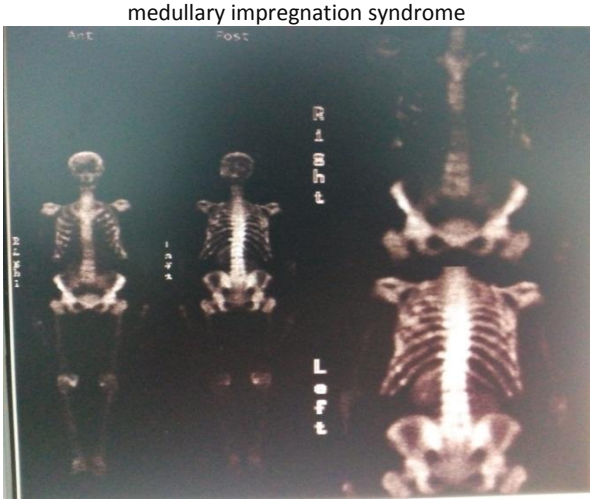


Figure 4: Whole body bone scintigraphy: low visualization of kidneys>> "super-scan" pattern pleading for secondary bone determinations with a possible paraneoplastic medullary impregnation syndrome



Oncology admission

The patient was referred to oncology and she started chemotherapy with capecitabine and zoledronic acid after stabilization. The oncologist further investigated the implication of the bone system through a whole body bone scintigraphy. The result pleaded for multiple secondary bone determinations with a possible paraneoplastic medullary impregnation syndrome. Three months after initiation of

appropriate systemic treatment for metastatic disease we see a partial recovery of hematopoietic function – her hemoglobin rose to 9 g/dl and platelets are around 90.000/μl .

COMMENTS

In conclusion, in front of a typical anemia that doesn't respond to typical treatment, as clinicians, we are obliged to search for additional causes and "cut the weed at the root".

The particularities of this case reside in the biochemical profile of the anemia – iron deficient at first encounter, then suggestive of Evans syndrome which delayed the diagnosis. In addition the ultrasonographic appearance of the breast tumor – more typical of a fibromatous degeneration did not raise suspicion at the first glance.

Breast cancer is the most common malignancy in women. Approximately 80% of women with metastatic breast cancer have skeletal metastases, which are often the result of bone marrow infiltration of malignant cells with subsequent progression and invasion of the skeletal cortex [1][2][3][4][5]. The most common complications of skeletal metastases are pathologic fractures, spinal cord compression as the result of vertebral compression fracture or extension of the tumor beyond the epidural space, and hypercalcemia [3][4][5]. Furthermore, skeletal metastases sometimes require surgery or radiation therapy to treat pain or an impending fracture. Bone marrow metastases result in the invasion and destruction of the bone tissue matrix by tumor cells [2]. Although bone marrow infiltration with micro metastasis is commonly present among breast cancer patients, total bone marrow infiltration – bone marrow carcinomatosis, BMC – resulting in profound pancytopenia is extremely rare.[2][6][7]

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Article received on January 12, 2015 and accepted for publishing on March 15 2015.

Case report – hepatocytolytic syndrome hiding mesenteric venous ischemia

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BACKGROUND

Acute mesenteric ischemia (AMI) is a syndrome caused by inadequate blood flow through the mesenteric vessels, resulting in ischemia and eventual gangrene of the bowel wall. AMI may be classified as arterial or venous, non-occlusive or occlusive. The overall prevalence of AMI is 0.1% of all hospital admissions [1]. The exact prevalence of mesenteric venous ischemia (MVT) is not known, because many cases are presumed to be limited in symptomatology and to resolve spontaneously.

CASE PRESENTATION

A 70 years old male patient presented to our gastroenterology department for investigating an acute hepatocytolytic syndrome (AST 297 UI/ml, ALT 616 UI/ml). He was non-smoker, with no history of hepatotoxic treatment or other toxic substance abuse. One month before he had normal liver enzymes at clinical tests (AST 50 UI/). He was asymptomatic on admission, with unremarkable physical exam: except sensitive abdomen in the right hypochondrium, moderate hepatomegaly (left liver lobe hypertrophy) and abdominal postsurgical scars. His blood pressure was 110/70 mmHg with no orthostatic changes. A regular heart rate of 90 beats/min and respirations of 18-20/min were observed.

From the patient history: he was operated six years ago for abdominal aortic aneurysm with reimplantation of both renal artery after aortic graft, plus aorto-iliac bilateral stent, he has diabetes mellitus type 2 (on oral antidiabetic drugs from 2008), hypertension, ischaemic heart disease, systemic atheromatosis, laparoscopic colecistectomy in 2011 and right coxartrosis. A control angiogram and Doppler ultrasound was performed six months ago, with no evidence of occlusion or blockage of the mesenteric arteries.

At presentation the patient had hepatocitolytic syndrome – elevated serum transaminases ALT 537 UI/L ; AST 297 UI/L, cholestatic syndrome – elevated alkaline phosphatase ALP 124 UI/L, no modification on hemograme, hyperglycemia 190 mg/dl with HbA1c 6.8 %, serum creatinine 1.3 mg/dl. We tested the patient for virus B and C infection, and we found Ac anti HVC positive. Then we performed HCV RNA viral load 115 UI/ml (low viral load) and supportive hepatic treatment was immediate conducted. On abdominal ultrasound we found: mild hepatomegaly, post-colecistectomy status, normal CBP and a left renal

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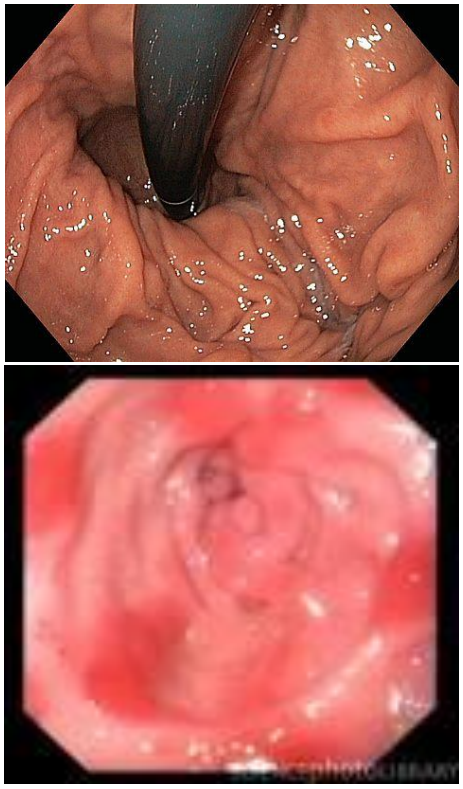
cyst.

After a few days the patient accused upper abdominal pain, nausea, fatigue, vomiting, constipation and oliguria. The blood pressure was 140/80 mmHg, the abdomen was symmetrically distended, dull to percution with absent bowel sounds with firm and tender to palpation in the mid-epigastrium. We did an upper GI endoscopy that revealed hiatal hernia, diabetic gastroparesis and duodenitis (figures 1a, 1b)

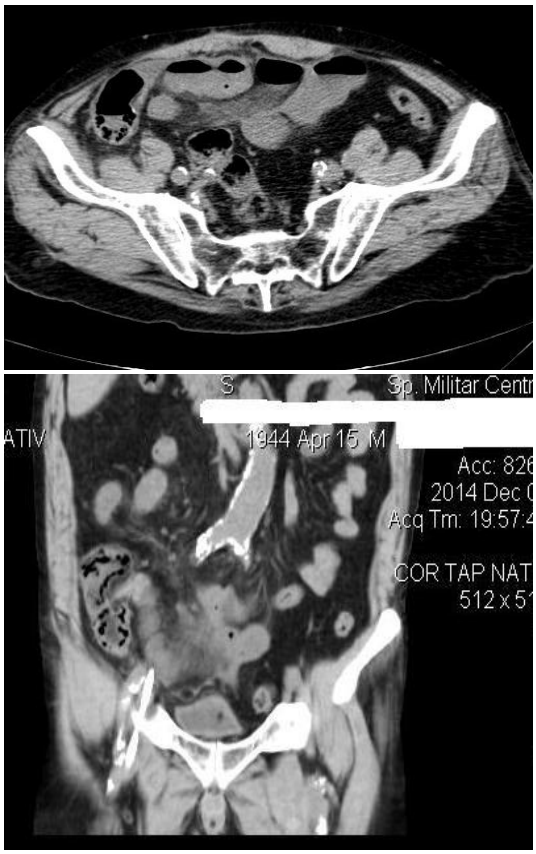
Figures 1 (a, b).



The laboratory tests showed normalization of liver enzymes, but rapid increase of urea 270 mg/dl and creatinine 4.88 mg/dl needing temporary hemodialysis in ICU, by femoral venous catheter placement. On abdominal examination, the patient had persistent abdominal pain, tenderness to palpation and increased abdominal wall rigidity. The native CT scan performed immediately (due to renal function impairment) did not confirmed arterial mesenteric ischemia suspicion, and also the biological markers D-dimers, CK, CK-MB where negative, with no signs of hypovolemia or hypotension.

After the temporary hemodialysis, another CT scan was performed, this time with contrast, because the suspicion of mesenteric ischemia remains, the patient had signs of peritonitis (right inguinal fossa pain and tenderness, exacerbated by moving the peritoneum, coughing and the Blumberg sign positive) and on ultrasound he has periappendiceal fluid collection and intestinal wall thickening. The contrast enhanced CT scan revealed: terminal ileum wall oedema, suggestive for acute mesenteric vein ischaemia, without lacunar imagine of a possible thrombus (arterial or venous) – figures 2a, 2b.

Figures 2 (a, b).



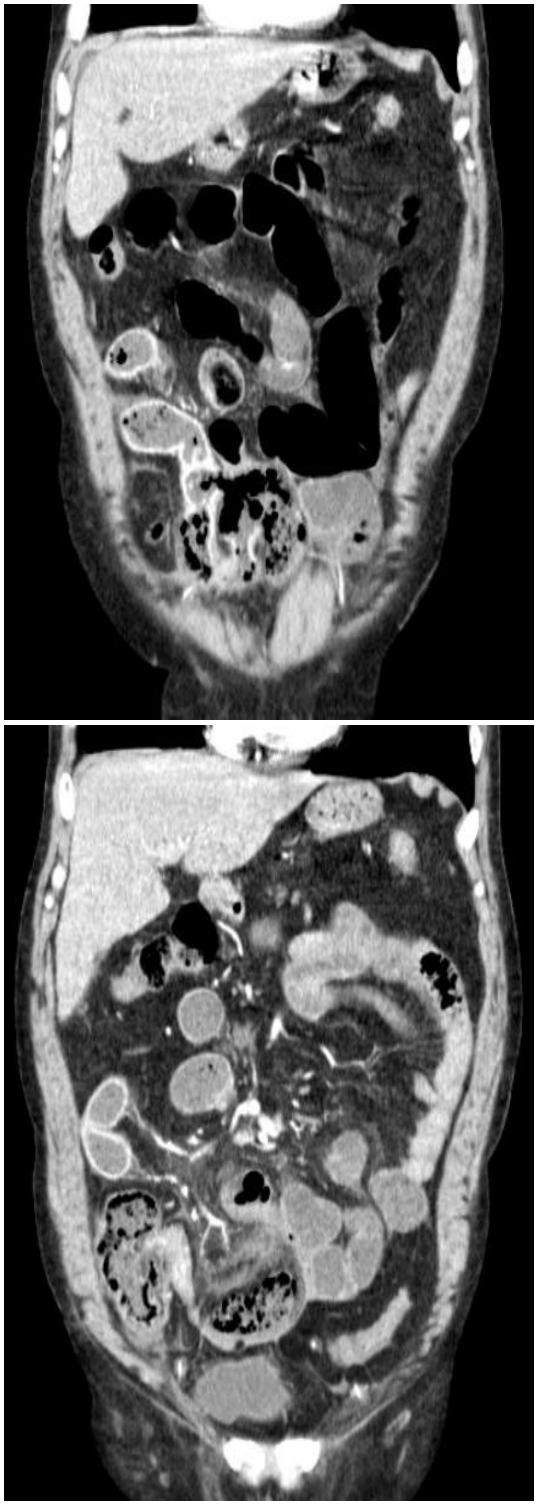
Initially the surgical intervention was temporized and the patient had conservatory heparinotherapy for 10 days (taking into account the patient comorbidities, history and risk factors for thrombosis), but the patient general status did not improved, he had nausea and vomiting, also persistent abdominal pain, fever and leucocytosis. We took blood cultures, remove central venous catheter and femoral venous catheters and continued antibiotherapy.

We did another CT scan with contrast one week later that showed distal ileum thickening with iodophile walls in arterial phase and necrosis, plus entero-enteral fistula localized in the right inguinal fossa (figures 3a, 3b, 4a, 4b).

Figures 3 (a, b).

The treatment included both surgical and anti-thrombotic therapy and we transferred the patient to the surgical department. A median laparotomy was performed, and mesenteric venous ischemia modifications were found at 5 cm from ileocecal valve, with entero-enteral fistula and cecal abscess. A large enterectomy was made, with resection of approximately 150 cm of the ileum (figure 5), followed by peritoneal cavity lavage and drainage, then protective right ileostomy. The postoperative evolution was favorable, and the patient was released with normal liver and renal function, with protective ileostomy. At three months the patient came in good clinical status, and ileostomy reversal was made. Further investigations are needed for this patient regarding hypercoagulability status or identifying other MVT risk factors, and of course close follow up for prompt diagnosis and treatment of recurrence.

Figures 4 (a, b).



Figures 5.

DISCUSSION

Mesenteric ischemia is caused by a reduction in intestinal blood flow, due to occlusion, vasospasm and/or hypoperfusion of the mesenteric vessels. The clinical consequences can be catastrophic, including sepsis, bowel infarction, and death, making rapid diagnosis and treatment imperative.

Injury severity is inversely proportional to the mesenteric blood flow and is influenced by the number of vessels involved, systemic mean blood pressure, duration of ischemia, and collateral circulation [1]. Mesenteric ischemia may be classified acute and chronic, based upon the rapidity and the degree to which blood flow is compromised.

Acute mesenteric ischemia refers to the sudden onset of intestinal hypoperfusion, which can be due to occlusive or nonocclusive obstruction of arterial or venous blood flow. Nonocclusive arterial hypoperfusion is most commonly due to primary splanchnic vasoconstriction [2].

Chronic mesenteric ischemia (also called intestinal angina) refers to episodic or constant intestinal hypoperfusion, which usually develops in patients with mesenteric atherosclerotic disease [2].

Venous thrombosis is predominantly a result of stagnation of blood flow, vascular injury, and hypercoagulability (ie, Virchow's triad) [3]. First differentiated from arterial causes of acute mesenteric ischemia 75 years ago, acute mesenteric venous thrombosis (MVT) is an uncommon disorder with non-specific signs and symptoms, the diagnosis of which requires a high suspicion rate [4].

Mesenteric vein thrombosis almost always involves the distal small intestine (superior mesenteric venous drainage) and rarely involves the colon (inferior mesenteric venous drainage) [5]. The anatomic site of involvement in acute mesenteric venous thrombosis is most often ileum or jejunum, followed by colon and duodenum [6].

Acute thrombotic occlusion of one or more mesenteric veins reduces perfusion pressure due to increased resistance in the mesenteric venous bed. As flow stagnates, increased venous pressure leads to efflux of fluid into the tissues, causing profound bowel-wall edema, which can lead to submucosal hemorrhage. If the venous arcades and vasa recta are involved and venous return from the bowel wall is completely occluded, bowel infarction will occur [4].

Causes of MVT include the following (>80% of patients with MVT are found to have predisposing conditions):

- Hypercoagulability [7] from protein C and S deficiency [8, 9] antithrombin III deficiency, dysfibrinogenemia, abnormal plasminogen, polycythemia vera (most common), thrombocytosis, sickle cell disease, factor V Leiden mutation, pregnancy, and oral contraceptive use [10, 11]
- Tumor causing venous compression or hypercoagulability (paraneoplastic syndrome)
- Infection, usually intra-abdominal (eg, appendicitis, diverticulitis, or abscess)
- Venous congestion from cirrhosis (portal hypertension)
- Venous trauma from accidents or surgery, [12, 13] especially portacaval surgery
- Increased intra-abdominal pressure from pneumoperitoneum during laparoscopic surgery [12]
- Pancreatitis
- Decompression sickness

The diagnosis is most often made by contrast-enhanced computed tomography, though angiography and exploratory surgery still have important diagnostic as well as therapeutic roles [4]. CT findings of intestinal ischemia due to acute MVT include wall

thickening > 3 mm in an adequately distended segment, abnormal enhancement patterns, distended luminal diameter, thickened mesentery, indistinct bowel wall margins, and the presence of new or unexplained ascites [14].

Thus, abdominal CT with adequate venous phase contrast should be used as the initial diagnostic test of choice for MVT, with angiography reserved for patients with non-diagnostic CT results yet a reasonable clinical suspicion for MVT (i.e. no alternate explanation for symptoms and either a known hypercoagulable risk factor or family history of venous thrombosis)[4].

Anticoagulation prevents clot propagation and is associated with decreased recurrence and mortality [4]. Immediate heparinization is currently accepted as standard therapy. Heparin has been shown to prevent recurrence of thrombosis after intestinal resection and to be associated with lower mortality when recurrence does occur [4].

When intestinal infarction is suspected by virtue of clinical decompensation or radiologic/CT findings, immediate exploration of the abdomen – either by laparotomy or laparoscopy is needed and non-viable segments of intestine are resected.

CONCLUSION

MVT is associated with a 30-day mortality of 13-15%. Without anticoagulant therapy, mortality increases, with a 25% recurrence rate. The combination of anticoagulant therapy with surgery is associated with the lowest recurrence rate (~3-5%)[1]. Although its mortality rate has fallen over time, acute MVT remains a life-threatening condition requiring rapid diagnosis and aggressive management.

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VARIA

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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, metaanalyses 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.

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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), available at 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;

(4) validates registered information;

(5) identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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The journal subscribes to the principles of the Committee on Publication Ethics (COPE).

MANUSCRIPT CATEGORIES AND SPECIFICATIONS

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).

Editorials

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 Military Med. Today (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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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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Abbreviations should be used sparingly and only where they ease the reader's task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

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The manuscript should be submitted in separate files: title page; main text file; figures.

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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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Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion, Acknowledgments and References*.

References

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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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Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

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SUBMISSION REQUIREMENTS

Manuscripts should be submitted online at daniel_costache@yahoo.com

A cover letter containing an authorship statement should be included.

The cover letter should include a statement covering each of the following areas:

1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.
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Accepted papers will be passed to production team for publication. The author identified as the formal corresponding author for the paper will receive an email, being asked to complete an electronic license agreement on behalf of all authors on the paper.

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Romanian Journal of Military Medicine
Vol. CXVIII, New Series, No 2/2015
ISSN 1222-5126