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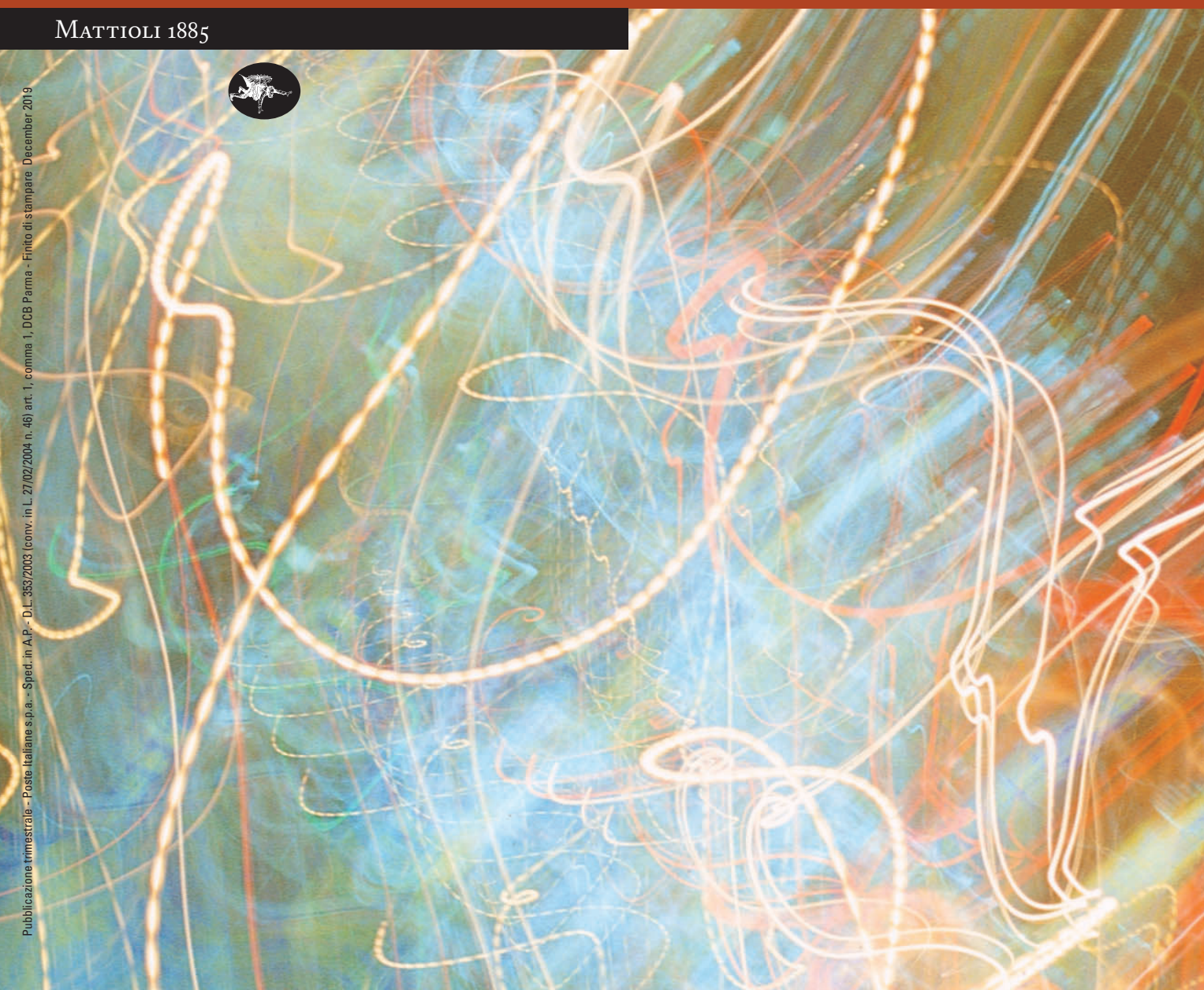

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Review

Conservative treatment in early stage endometrial cancer: a review

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Summary. Endometrial Cancer (EC) is the commonest gynecological cancer and its incidence is increasing. The diagnosis of endometrial carcinoma in young women of childbearing age is rare. Indeed, only 4% of patients with endometrial carcinoma are <40 years of age. It's typically diagnosed in postmenopausal women. The standard approach for the management of endometrial cancer in young women of childbearing age is hysterectomy and bilateral salpingo-oophorectomy with or without lymphadenectomy but is not ideal for women interested in future fertility. We reviewed the published literature to clarify in fertile women who have not yet fulfilled their desire for motherhood, what are the strategies, the risks of a conservative treatment of early stage of Endometrial Cancer and what are the obstetric outcomes in this patients. Recently, several studies have reported encouraging results on fertility-sparing management of EC with high dose of progestins in selected women associated or not with hysteroscopic resection. (www.actabiomedica.it)

Key words: endometrial cancer, fertility sparing surgery, conservative treatment, hysteroscopic resection uterine preservation

Introduction

Endometrial cancer (EC) is one of the most common gynecological cancers. More than 90% of cases of endometrial cancer occur in perimenopause women and 25% are premenopausal (1, 2). However, 4% of women with endometrial cancer are younger than 40 years old and over 70% of them are nulliparous at diagnosis, due to the fact that in the current era women delay their childbearing. The majority of endometrial cancers are diagnosed early stage (80% in stage I), with 5-year survival rates over 95%. Most endometrial cancer cases are sporadic, with only 10% considered familiar.

Endometrial carcinoma has been classified into two main clinic-pathological and molecular types: Type I and Type II.

Type I is the endometrioid type (EEC) (3) because it's similar to the endometrium and is characterized by genetic predisposition (eg. Lynch syndrome-LS), such as obesity, polycystic ovarian syndrome (PCOS), anovulatory cycles, irregular menstruation that causes hyper estrogenic state, that is a main predisposing factor for developing Type I EC. Type I EC has a favorable outcome due to minimal myometrium invasion (4).

Type II cancers are associated with higher patient age, high stage and grade, non-endometrioid histology, and poor prognosis, instead. It includes several subtypes such as serous, clear cell and undifferentiated carcinomas (5).

Most patients with endometrial cancer have an excess of estrogen and typically show a characteristic clinical profile: high body mass index (BMI) that is considered as overweight (BMI 25-30) or obese (BMI 30), often with other components of metabolic syndrome (hypertension, diabetes) (6). This is the most commonly identified risk factor because obesity is as-

sociated with peripheral estrogen conversion via aromatization in adipose tissue (7, 8). Nulliparity and infertility are classical risk factors for endometrial cancer. Other risk factors include unopposed estrogen therapy, estrogen-producing tumors such as ovarian granulose, theca cell tumors and early menarche/late menopause. Studies also show that exposure to tamoxifen increases the risk of endometrial cancer-related estrogen as well as an unbalanced hormone replacement therapy (9). Only endometrial carcinoma type I may be subject to a fertility sparing treatment.

Materials and methods

We performed a Pubmed, Medline search of articles published in English between 1959 and 2018 with the key words 'Endometrial cancer', 'fertility sparing surgery', 'conservative treatment', 'hysteroscopic resection' and 'uterine preservation'. Moreover, we identified several articles from bibliographies of these publications including case reports, case series, original articles, review articles, and meta-analyses, with the purpose of analyzing the different methods of treatment reproductive outcomes and follow-up after fertility sparing treatment in women with an early stage of EC.

Selection of patients: stage, grade and histopathology

When considering a conservative management approach, we should consider clinical and pathological characteristics of the tumor for can select the appropriate medical intervention. A conservative management approach could be considered in patients: <40 years old (relative indication), have to intent to preserve fertility and plan to conceive as soon as possible after remission, with no contraindication for medical treatment and with a histological diagnosis of grade I endometrial carcinoma; histotype: endometrioid with positive hormone receptor (tipe I), tumor diameter <2.0 cm, stage IA without myometrial and adnexal involvement, negative lymph-vascular space invasion (LVSI) and diffuse immunohistochemical expression of progesterone receptors on endometrial biopsy. These patients are considered as " low risk" population. According to Gynecologic Oncology Group (GOG) and Federation International of Gynecologic and Obstretric (FIGO),the most important prognostic factors (10) for lymph node metastasis in patients with EC were the grade of tumor and the depth of myometrial invasion with the risk of involvement less than 1% and excellent 5-year progression-free survival of 95% if the tumor is grade 1 with an overall survival of 90%. In the absence of risk factors, a conservative approach to surgical staging is feasible, safe and not associated with an increase in cancer-related mortality (11).

Diagnosis

Diagnosis should be performed by Hysteroscopy and endometrial biopsy (12, 13). The Society of Gynecologic Oncology (SGO) recommends that the preferred tissue formats include curettage and biopsy and that devices that result in crushed, cauterized, or very small samples are unacceptable (14). Imaging performed by MRI or Transvaginal Ultrasound by experts, helpful to detect possible myometrial invasion and exclude synchronous ovarian tumor or suspicious lymph-adenopathy. Seems that MRI is slightly more sensitive than ultrasound for the evaluation of myometrial invasion and that the implementation of both techniques increase sensitivity (15-16).

Selection of drug, dose, length of treatment

The standard treatment for EC is a hysterectomy with bilateral salpingo-oophorectomy, with or without lymph node dissection with pelvic washing, sometimes combine with adjuvant chemoterapy or radiotherapy is necessary. Although this is a highly effective approach, carrying a 5-year survival rate of 93%, it also results in a permanent loss of reproductive potential which is often unacceptable to younger women who wish to preserve their fertility. Therefore, selected patients with EC, are candidates for a conservative approach (2). Hence, fertility-sparing treatment with progestin is a good compromise for these women. Recently, hysteroscopic resection in addition to hormonal therapy followed by pregnancies in young women have been reported (17, 18).

Medical treatment

Conservative management of EC is based principally on medical treatment with oral progestins. Medical treatment Hormonal therapy, alone or in a combination with hysteroscopic ablation. Progestins (19, 20) are medroxyprogesterone acetate or megestrol acetate. Although today there is no consensus on the optimal dosage or duration of treatment, it appears that 62-75% of these women respond well to progestational treatment and the absence of progesterone receptors (PR), can make inhomogeneous the success of progestin treatment (2, 21). First Kistner in 1959 (22) to use for EC a progesterone formulation using various dosing strategies. Another method of treatment is Levonorgestrel-releasing IUD (23-25), removing the IUD when patients are ready to attempt pregnancy. With or without GnRH analogues, or a combination of IUD and oral progesterone (26). With a high response rates in patients with grade I EC, despite some patients with prior progesterone treatment.

Surgical treatment by hysteroscopy

Mazzon et al (17) reported a series of patient treated with hysteroscopyc resection of tumor with resection of the adjacent endometrial margins and the myometrium underlying the tumor with biopsies of uterine cavity and under general anesthesia followed by oral therapy with progestin, Megestrol acetate (160 mg daily) or Medroxyprogesterone acetate (400 mg/day), beginning the fifth day after the surgery and continuing for six months (27). Other authors describe the use of IUD for 12 months. Shan et al. instead performed hysteroscopy curettage during which resected the major part of tumor tissue, later followed by complete endometrial resection with hysteroscopy. Marton et al. performed an initial polypectomy resulted in the diagnosis of carcinoma. One month later performed a complete endometrial ablation (29). Park et al (28, 29) described the possible adverse effects of hysteroscopy resection prior to hormone therapy with an increase in adhesive syndrome after resection. This can influence the obstetric outcome.

Follow-up

In order to assess response, hysteroscopy and imaging at 6 months must be performed and not before. If no response is achieved after 6 months, standard surgical treatment should be performed. In case of complete response, conception must be encouraged and referral to a fertility clinic is recommend. Maintenance treatment should be considered in responders who wish to delay pregnancy. Patients not undergoing hysterectomy should be re-evaluated clinically every 6 months. After completion of childbearing, a hysterectomy and salpingo-oophorectomy should be recommended (30, 31). The preservation of ovary can be considered depending on age and genetic risk factors. Hence, the follow-up of these patients under conservative treatment in the first year included serial transvaginal Ultrasonography (TV-US) every three months. A computed tomography scan (CAT) six months after surgery is recommended. Several authors performed check-ups with only TV-US or in association with CAT scans, every six month starting from the second years. A strictly follow-up during the period treatment is recommended (21, 32). Hysterectomy should be recommended as the definitive treatment for patients with persistent disease. The need for bilateral salpingo-oophorectomy depends on the risk factor and therefore this possibility must be discussed with the patient. Patients who after 6 months of treatment have a partial response could be offered continuation of treatment for another 3 to 6 months.

A recent study concerning the use of antidiabetic drug metformin and its effect on EC cells has shown that metformin suppresses EC cell growth (associated with the reduction of the proliferation marker Ki-67) (33, 34) and have an anti-proliferative effect in women with EC and insulin resistance or PCOS (35). In the future a treatment could be possible in association with progestin and active weight management early stage of EC (36).

In premenopausal women who are obese, the use of aromatase inhibitors in adjunct to oral progesterone, initially Megestrol acetate 160 mg/day for 6 months subsequently as second treatment after second biopsy with persistent aypical hyperplasia or Grade I endometrioid endometrial cancer is added to Meges-

trole acetate 160 mg/day Anastrozole 1mg/day for 6 months or intrauterine device for 8 months if at the second biopsy the result was Grade III endometrioid endometrial cancer (37, 38), seems like a reasonable therapeutic option, as they have a significant proportion of their estrogen production coming from peripheral conversion in adipose tissue. Agorastos et al. in 2005 (39), demonstrate that aromatase inhibitors reduce endometrial thickness in patients who cannot be subjected to hysterectomy.

Strategies of fertility preservation

One of the strategies to preserve fertility is ovocyte cryopreservation. Patients with previous history of infertility or other risk factors for infertility should be encouraged to the use of assisted reproductive techniques. Reassuring data confirm the safety of Assisted Reproduction Techniques (ART) (40). Ovulation induction does not appear to be associated with increased risk of relapse, and subsequent pregnancies do not worsen oncological outcomes (41-43).

Outcomes

Primary outcomes are the evaluation of complete response to therapy, defined as the absence of disease on subsequent endometrial biopsy; we define partial response if the disease was downgraded to complex atypical hyperplasia. No response, defined as who have no evidence of response and progression is defined the presence of a higher grade of cancer on biopsy. Secondary outcomes are obstetrical outcomes (44, 45).

Pregnancy rate described in literature for exclusively hormonal treatment is between 35-60%, but after a combined treatment, hysteroscopic and hormonal pregnancy rate increases to about 70%. The recurrence rate of EC after conservative treatment is between 30-40% in from 4 to 66 months (44, 46, 47) and can be offered a re-treatment with progestin.

The mortality associated with conservative treatment of early stage of EC is very low despite the fact that the rate of recurrence is high (48).

Conclusion

Uterine preservation is a safe and feasible option in select young women who have not yet fulfilled their desire for motherhood with stage IA low-grade progesterone receptor positive endometrioid tumors with no metastatic involvement or risk factors. An adequate evaluation and a correct diagnosis by expert physician confirming the absence of myometrial invasion. The treatment of choice is progestin associated or not to hysteroscopy with a monitoring for long periods. Hysteroscopic surgery prior to hormone therapy may improve the rate of recurrence when the resection margins are free, although serve further studies. Individualization of care is important as each patient has different characteristics as well as different needs and expectations.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

Clinical and radiological features of mesenteric panniculitis: a critical overview

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Summary. The mesenteric panniculitis is a rare form of inflammation that mainly involves the mesenteric adipose tissue. The etiology remains unknown and the disease has been associated with various conditions such as cancer, abdominal trauma, previous surgery, autoimmune diseases and obesity. Mesenteric panniculitis can be divided into two main groups: the mesenteric panniculitis with only the inflammation and degeneration of the mesenteric fat, and the retractile panniculitis, mainly fibrotic, with retraction of the surrounding structures. From a radiological point of view, there are two main signs: the fat ring sign, which is the presence of normal fat around vessels and lymph nodes, and the pseudocapsula around the lesion. In this paper, we present the imaging and clinical features of mesenteric panniculits with particular reference to the differential diagnosis and the possible etiological associations. (www.actabiomedica.it)

Key words: mesenteric panniculitis, CT, MRI, chronic inflammation of the mesentery

Introduction

The mesenteric panniculitis is a rare and chronic inflammatory disease which involves primarly the mesentery and rarely other sites like omentum and mesocolon. The etiology of the disease remains understood but it recurs in association with various conditions like abdominal surgery, mesenteric ischemia, trauma, obesity, abdominal and extra-abdominal cancer and abdominal inflammatory disease. The disease is often asymptomatic but it may produce symptoms like abdominal pain, constipation or diarrhoea, and dyspepsia. Sometimes it appears as an abdominal mass and may produce bowel obstruction or ischemia, requiring urgent surgery. The two main form of mesenteric panniculitis are: the classical type with inflammation, necrosis and degeneration of fat, and the retractile panniculitis with a prominent fibrosis of mesentery with rectraction of the surrounding structures. The diagnosis is mainly radiological, and the computed tomography (CT) and magnetic resonance imaging (MRI) are the most useful methods to detect the disease. To assess the diagnosis, in general, the radiologist must consider some morphological manifestations like the presence of a circumscribed mass in mesentery with a fibrotic pseudocapsule, vessels and lymph nodes inside the mass and a preserving rim of fat around them, the so-called fat ring sign. However, the mass with a prominent fibrotic component with o without retraction of the surrounding structures may appear as a solid mass.

In this paper, we aim to review the clinical and radiological manifestations of the disease on the basis of our direct experience and the literature data. Indeed, we want to present some ideas on the etiology of this disease.

Clinical manifestations and associated disorders

The mesenteric panniculitis is a non specific inflammatory process of mesentery which produces

degeneration, necrosis and fibrotic proliferation of mesenteric fat (1). The mesenteric panniculitis rarely involves the omentum, the peritoneum of the large intestine, the retroperitoneal and pelvic fat (2). It is more common in men, with a male-to-female ratio of 2-3:1, and it is more common in Caucasian men (3). The disease is more frequent between the 6th and 7th decades of life (4), but some pediatric cases were described (5). The mesenteric panniculitis is a rare disease and the prevalence reported in the scientific literature ranges between 0,16-3,3% (6).

The mesenteric panniculitis is usually asymptomatic and when symptomatic, the clinical manifestations are not specific, depending on the size and location of the mass and its relationship with the bowel, vessels and lymphatics (7). However, the mesenteric panniculitis may be the cause of abdominal pain, nausea, vomiting, diarrhoea, constipation, fever, weight loss, chylous ascites (8). In a recent clinical study, the major complaint was abdominal pain, which was present in the 72% of patients with mesenteric panniculitis. Other mesenteric panniculitis related symptoms were nausea and vomiting and diarrhoea (9). Akram et al. reported in a case series of 92 patients that the most common symptoms were abdominal pain (70%), bloating and distention (26%), diarrhoea (25%) and weight loss (23%). Only 10% were asymptomatic at the time of diagnosis (10). In a study of Nyberg et al. 5 of the 27 patients were asymptomatic, 13 had symptoms without signs of systemic inflammation, five were symptomatic with signs of systemic inflammation and four had severe disease with multiple hospitalisation, chronic, refractory or complicated disease. However, the 4 patients with high clinical score had a concomitant chronic disease (Bechet's, Crohn's, psoriasis arthritis and hereditary spastic paraparesis) and for 3 of them, the concomitant disease caused the major morbidity. In the same study the abdominal pain was reported by 21 patients and was the most common symptom. Six patients specifically reported symptoms at night and symptoms related to body posture. In addition, nausea, weight loss, flatulence and diarrhoea were reported. Tenderness in the left hypochondrium and sometimes a tender palpable mass were described. Most symptomatic patients had chronic discomfort but some patients had acute episodes with intense pain (11).

In mesenteric panniculitis patients, the blood tests results are often normal. The erythrocyte sedimentation rate and C-reactive protein level may be elevated in response to the inflammatory state and may serve as markers of response to medical therapy (8). The course of the disease is usually benign but sometimes the mesenteric panniculitis may be the cause of bowel perforation, bowel occlusion (small bowel obstruction was present in 24% of patients in the study of Akram et alt.), bowel ischemia and renal failure due to ureteral stenosis and may be therefore a potentially lethal disease requiring urgent medical or surgical treatment (10, 12, 13). The correct evaluation of the symptomatology, therefore, appears to be a fundamental step in the clinical and therapeutic framework of this disease to identify the most critical situations.

The mesenteric panniculitis is a disease with an understood etiology but, in the scientific literature, it was associated with various conditions such as mesenteric thrombosis, mesenteric arteriopathy, autoimmune disease, such as IgG4 related disease, pancreatitis, urinary system diasease and infection (14, 15). Other factors, such as urinary gallstones, abdominal aortic aneurysm, prior abdominal trauma, peptic ulcer, or chylous ascitis, have also been linked to this disease (16).

However, the most common conditions associated with mesenteric panniculitis are the abdominal surgery and cancer (3). The most common types of cancer associated with mesenteric pannicultis are the abdominal lymphoma, melanoma, colon carcinoma and prostate cancer (3, 17). Various studies have attempted to understand the correlation between mesenteric panniculitis and cancer. In particular, a possible paraneoplastic nature of mesenteric panniculitis was assessed, but this association is widely discussed. In an analysis by Gögebakan Ö et al., the authors rejected the hypothesis of a paraneoplastic etiology of the disease (18). Buchwald carried out a cohort study in which no statistically significant association was found between the course of mesenteric panniculitis and cancer, considering the pathology as an epiphenomenon and not as a true paraneoplastic event. In fact, one of the reasons why CT and MRI are most frequently required is neoplastic disease, which could explain the high incidence of mesenteritis in cancer patients (19). However, in the Van Puttie study, a significantly higher prevalence of

neoplastic disease was found in patients with mesenteric panniculitis (20). The same authors reported an increase in the prevalence and risk of future cancer during a 5-year follow-up (20), while Scheer described a five-fold higher risk of malignancy in the presence of mesenteric panniculitis (21). It has also been proposed that mesenteric panniculitis may be caused by the ischemic or inflammatory effect of chemotherapy (22). Among tumors, is interesting the association with lymphomas. Various authors have hemphasized the correlation between abdominal lymphomas, which is the most frequent neoplasia with an intraperitoneal and extraperitoneal localization, and the mesenteric panniculitis. In a study of Khasminsky et al., the authors, on a total of 166 patients who were diagnosed with non-Hodgkin lymphoma over a period of 5 years, found a prevalence of mesenteric panniculitis among patients with lymphoma of 1.8%, which corresponds to the range of its prevalence in the general population (23). However, Coulier B. et al. reported that the abdominal lymphoma occurs in the 15,1% of patients with a history of cancer while in the experience of Daskalogiannaki M. et al. the association between mesenteric panniculitis and lymphoma was found in 25 of 88 patients. Interesting, Fatahi Bandpey et al. reported that 16 (33%) of the 48 analyzed patients with abdominal lymphoma showed mesenteric panniculitis: 7 of them (43.75%) at the time of diagnosis and the remaining 9 patients (56.25%) later. Of the 9 patients who developed mesenteric panniculitis during the disease evolution, R-CHOP (55.55%) and ABVD (33.33%) were the most frequent regimens associated with the onset of the diasease. Of the 7 patients with the mesenteric panniculitis at the diagnosis, the follicular non-Hodgkin lymphoma (42.9%), and the Hodgkin lymphoma (28.6%) were the most common lymphoma types associated with the mesenteric panniculitis (4, 15, 24).

The most common surgical treatments associated with mesenteric panniculitis are the cholecystectomy, appendicectomy, hysterectomy and colectomy (25). Emory reported a series in which 84% of patients had a history of trauma or abdominal surgery (8). Durst et al. stated that the recent surgery was related to 17% of its cases, constituting a predisposing factor (25, 26). In recent reports the mesenteric panniculitis was observed in patients treated with bariatric surgery. In these patients the mesenteric panniculitis was observed after surgery and the disease may be considered a truly surgical complication (27, 28).

In addition, Mahafza et al. reported a prior history of abdominal surgery in 44 (49%) of the 90 patients with MP of whom 9 (10%) had more than one abdomino-pelvic surgery. The prevalence of the disease in patients with a history of previous abdominal surgery was 9.2% (44/476) while it was of 1.1% (46/4282) in patients without prior abdomino-pelvic surgery, a statistically highly significant difference (p=0.0001) (29).

In our clinical experience, the mesenteric panniculitis was associated with abdominal cancer, mainly lymphoma, colon cancer and genito-urinary system cancer, abdominal surgery, urinary lithiasis and abdominal inflammatory conditions, like reported in literature.

At this point it is interesting to discuss these possible etiological correlations. The mesenteric panniculitis is a chronic inflammatory disease of the mesentery whose cause is little known today. If it is true that mesenteric panniculitis is occasionally diagnosed with radiological methods and that most radiological computed tomography and magnetic resonance examinations of the abdomen are carried out in patients with a history of cancer or abdominal surgery, for which mesenteric panniculitis could be a simple epiphenomenon without any etiological correlation with the aforementioned conditions, it is also true that mesenteric panniculitis is rarely diagnosed in patients who have not a known pathology causing mesenteric inflammation. In fact, it is reasonable to think that benign or malignant conditions such as neoplasms, abdominal surgery, inflammatory processes of the abdomen, can cause a chronic fibro-inflammation of the mesentery. In these cases we could speak of secondary forms of mesenteric panniculitis. This could have important repercussions in the diagnosis and treatment of the disease because abdominal symptoms secondary to chemotherapy, radiotherapy, abdominal surgery or abdominal inflammatory process could still suspect an underlying mesenteric panniculitis, as a subacute or late manifestation or complication. This reasoning does not arise only from the experience of the authors but also from the

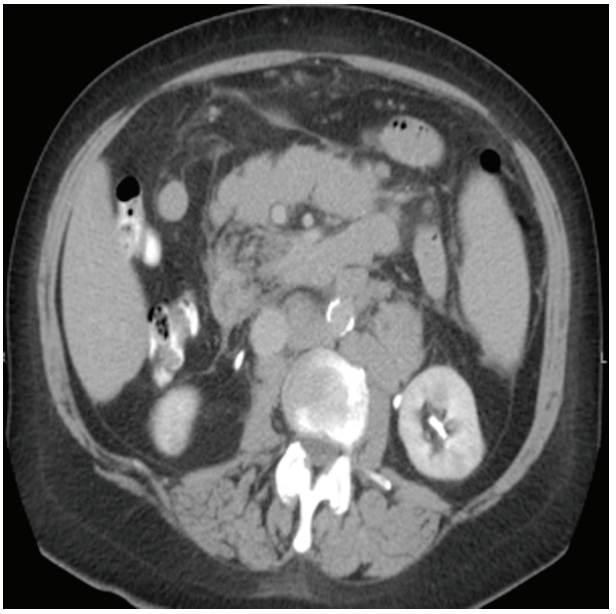

Figure 1. A case of abdominal lymphoma. The CT image shows multiple, large bulky masses in the abdomen due to coalescent lymph nodes

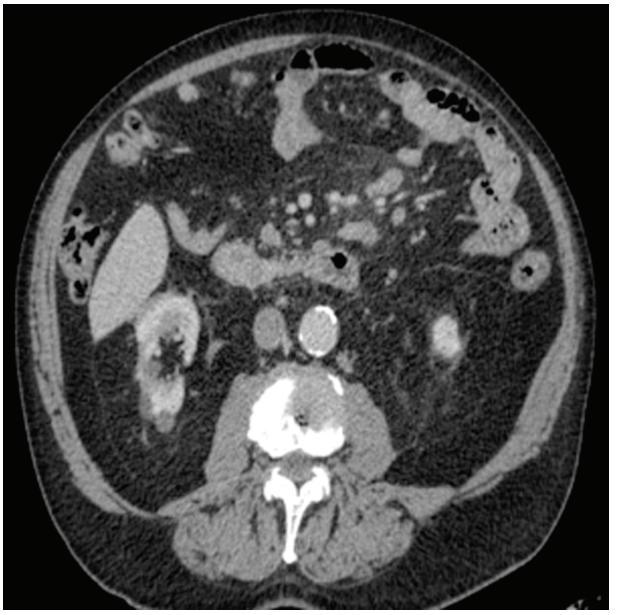

Figure 2. In the same patient of figure 1, the CT shows the presence of a mesenteric mass after the treatment for lymphoma. The lesion was stable at subsequent controls and this mass could be considered a secondary mesenteric panniculitis

literature. The already exposed correlation with radiochemotherapy in tumors could be an example such as the rapidly progressive and aggressive panniculitis forms found after bariatric surgery in two cases or the IgG4 found in the histological specimens of mesenteric panniculitis associated with IgG4 related disease (24, 27, 30, 31) (Figure 1-2).

Treatment

About the treatment, there is no consensus among the authors. The choice of treatment depends on the symptomatology. If the disease is asymptomatic, medical treatment is usually not carried out. If the disease produces abdominal symptoms, usually abdominal pain or dyspepsia, medical treatment is the first choice to reduce the symptomatology and/or to produce a regression of the disease. When the mesenteric panniculitis is complicated by intestinal obstruction, ischemia or perforation, or when the medical treatment fails, the mesenteric mass and/or the adjacent bowel may be removed. Surgery should, however, only be performed in patients who really need it because it involves the removal of the mesenteric mass and the bowel affected by the pathological process. In fact, the surgical removal can be difficult due to the extent of the disease and the vascular obstruction of the mesenteric vessels. We must also consider the extension of the intestinal tract involved, because the removal of a long portion of small intestine can expose these patients to short bowel syndrome. Therefore, in the absence of the aforementioned requirements that make the patient eligible for surgery, the surgical approach should be limited to performing a biopsy.

Regarding the medical therapy, the most common drugs used are the tamoxifen, prednisone, colchicine, azathioprine and other immunosoppressive drugs, and thalidomide. In particular, corticosteroids have been used as first-line therapy while immunosuppressive drugs have been used as second-line therapy in cases of symptomatic recurrence or if the first line therapy failed. However, Sahin et al. reported a good clinical response in patients with mesenteric panniculitis, unrelated with other clinical conditions, treated with NSAIDs and/or antibiotics, that, in these patients,

could indicate that the mesenteric panniculitis is of infectious origin. The response of the disease to treatment and its course is usually good and the progression is rare. In the study of Akram et al., 44 symptomatic patients of the 92 considered in the study received a treatment. Treatment included medical therapy alone in 26% of patients, surgery alone (partial or complete resection of the mesenteric mass and adiacent small bowel) in 13%, surgery followed by medical therapy in 9% of patients. Ten percent responded to surgery alone, 20% responded to medicl treatment and surgery, and 38% responded to medical therapy alone. The progression of the disease was observed only in 6 patients who received only the medical therapy.

Daskalogiannaki et al. reported the radiological stability of the disease in 20 of 21 mesenteric panniculitis patients during a follow-up period between 5 months and 3 years. Of these patients, 18 had an underlying malignancy and the mesenteric panniculitis was not treated; three patients without malignancy received a medical treatment with a clinical response in two patients and intermittent symptoms persisted in the third patient but the mass remained the same on CT images. In one patients, the authors observed a slight increase in the size of the fatty mass with mild thickening of the stripe, which was interpreted as a progression of the disease. Issa et al., reported the cases of two patients with symptomatic mesenteric panniculitis treated with prednisone with complete clinical and radiological response. The study of Buchwald et al. showed that mesenteric panniculitis was stable in 80.9% of patients and regress in the 19,1% of patients on CT follow-up.

In general, the mesenteric panniculitis is often asymptomatic and it not require a treatment and remain stable. The treatment is reserved for patients who have abdominal symptoms and the response to treatment, in our opinion, must be assessed with clinical and radiological scores. The response to treatment appears to be releated with the presentation of a symptomatic mesenteric panniculitis: if the disease shows a slight abdominal symptomatology the prognosis is good as well as the clinical response to therapy whereas worst outcomes and death may occur in patients with acute abdominal complications requiring urgent surgery (3, 10, 15, 19, 25, 27, 32-35).

Imaging

The radiological features of the mesenteric panniculitis are well known in medical literature and the radiological examinations are the keys to identify the mesenteric panniculitis. In most studies, the mesenteric panniculitis has been described on CT images. The mesenteric panniculitis is usually described as a mass heterogeneous, solitary, localized mainly in the mesentery, usually left oriented, with delocalization of the small intestine and surrounding structures. The two main radiological signs are the fat ring sign, described as a ring of normal fat around vessels and lymph nodes and the pseudocapsule sign, defined as a thin, usually <3 mm, fibrotic rim around the mass. These two signs are considered the radiological hallmarks of the mesenteric panniculitis. They recur in 70%-92% and 50%-60% of the patients, respectively. The mesenteric vessels often appear engulfed and some cases of mesenteric vessels thrombosis have been described (Figure 3-4). ystic components have also been described and may be the result of lymphatic or venous obstruction as well as necrotic change (33, 36, 37). Calcifications may be present, often in the necrotic portion of the mass. The mesenteric panniculitis is a disease of the mesentery, but it may involve the pancreatic region and the porta hepatis (33), the omentum and other part of the peritoneum such as the mesocolon (38, 39).

The mesenteric panniculitis is a chronic inflammatory disease of mesentery with a variable fibrotic component that evolves from the chronic inflammation of the mesenteric fat and may produce a retraction of the surrounding structures. In this case, we should use the term of retractile panniculitis.

About this, three progressive pathological stages of mesenteric panniculitis have been proposed in literature. In the first phase the mesenteric fat undergoes a process of diffuse degeneration (lipodystrophy panniculitis), which results in an inflammatory form (mesenteric panniculitis) and finally in a fibrotic form with retraction of the intestine and surrounding structures (retractable panniculitis). However, some authors used only the term mesenteric panniculitis to indicate the degenerative-inflammatory form and the term retractable panniculitis to identify the fibrotic form complicated by retraction of the surrounding structures (8, 15). This di-

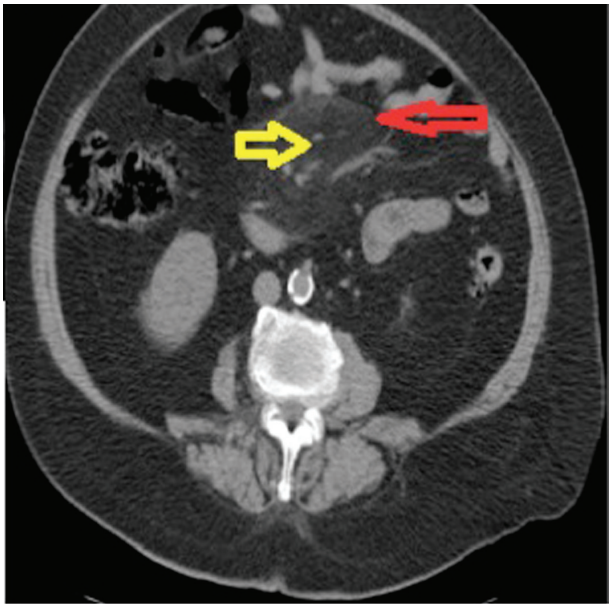

Figure 3. An axial CT image shows a mesenteric panniculitis with a fibrotic band around the mass (pseudocapsule sign/red arrow) and a rim of preserved fat around vessels and lymph nodes (the fat ring sign/yellow arrow). The mesenteric vessels are engulfed

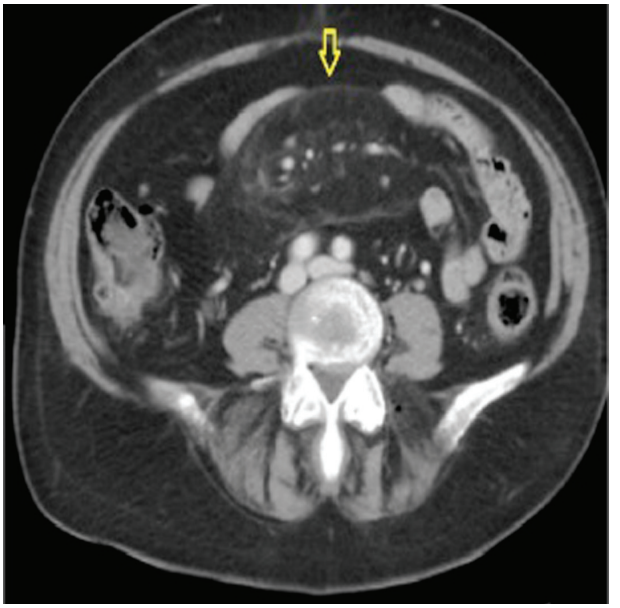

Figure 4. The axial contrast enhanced CT image shows a large mesenteric mass with a pseudocapsule (yellow arrow), lymph nodes and vessels inside the mass and displacement of the surrounding bowel

vision is, in our opinion, very important. The retractile form may be quite different from the "classical" mesenteric panniculitis also on images. The fibrotic component may be variable and the rectractile form may have the appearence of a mass with a partial fibrotic component or a predominantly solid and fibrotic mass without the pseudocapsule and fat ring sign. The fibrotic component may also cause the infiltration of the intestinal wall and perforation of the viscera (Figure 5-6).

Indeed, in one case in literature, the mesenteric panniculitis appeared as a multiple, fibrotic masses in the peritoneal cavity, mimicking a peritoneal carcinomatosis (40).

At this point, we must remember the usefulness of imaging to identify the complications of mesenteric panniculitis. In fact, the radiological methods allow to define the extent of the disease and the possible occlusive, perforative and ischemic intestinal complications more frequently related to the fibrotic infiltration or traction. In this context, the correct radiological evaluation of the pathology and the relationships with neighboring structures is fundamental for a correct therapeutic planning.

In the medical literature the radiological features of mesenteric panniculitis are described mainly on computed tomography images. Few are the articles in which the mesenteric panniculitis is described on magnetic resonance images. In general, the intensity of the signal at MRI varies depending on the histological components and stage of the disease. The inflammatory form of mesenteric panniculitis is usually hypointense in T1 weighted images and hyperintense in T2 weighted sequences. When the fibrosis is predominant, the disease appears as a localized mass of fibrous tissue, hypointense in both T1 and T2 sequences. The pseudocapsule appears usually as a hypointense sharp around the mass. Delayed contrast enhancement is characteristic and indicates the presence of fibrous tissue (39, 41) (Figure 7-8).

In our experience the mesenteric panniculitis on MRI images appeared as a mesenteric mass hypointense in T1 and hypointense to hyperintense in T2 sequences. The pseudocapsule appeared as a hypointense fibrotic sharp around the mass. The best sequence to detect the mesenteric panniculitis was the fat saturated T2 sequences in which the mesenteric panniculitis was

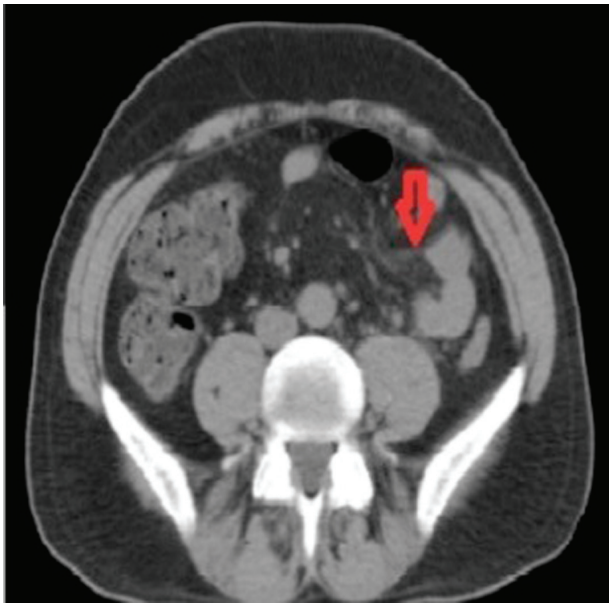

Figure 5. An axial contrast enhanced CT image shows a mesenteric mass with a fibrotic portion with mild retraction of small bowel (red arrow). The mass is compatible with the retractile form of mesenteric panniculitis

Figure 6. The CT image shows a retractile panniculitis. In this case the fibrotic portion of the mass cause an important retraction on the adiacent small bowel (red arrow). The description of small bowel retraction due to fibrotic mesenteric panniculitis is the most important aspect of the disease because it can influence the prognosis and treatment of the disease in relation to the symptomatology of intestinal obstruction

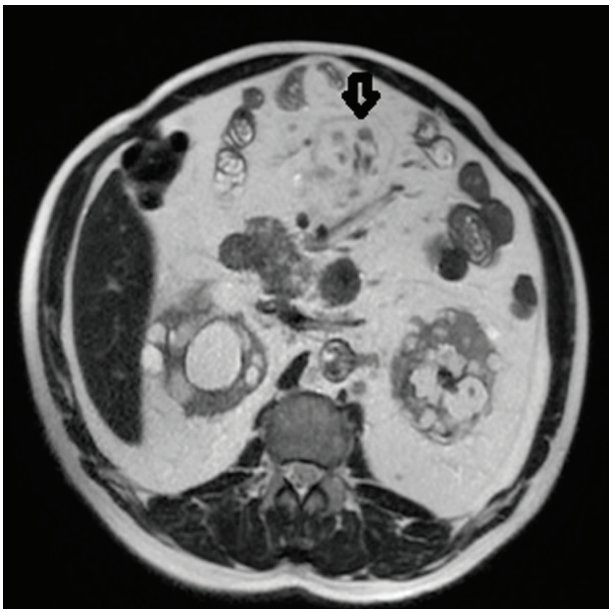

Figure 7. An axial T2 weighted image showed the presence of a mass with a hypointense pseudocapsule (black arrow). Note the enlarged lymph nodes inside the mass

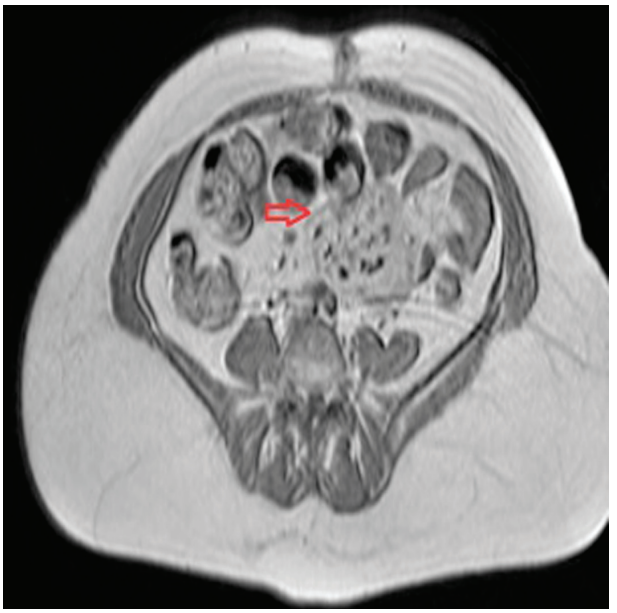

Figure 8. An axial T1 weighted image shows the presence of a hypointense mesenteric mass with the classical hypointense, fibrotic pseudocapsule around the mass (red arrow). The fat around vessels and lymhp nodes within the mass has the same intensity of the normal fat (the fat ring sign)

easily identified after the saturation of the mesenteric fat as a high signal mass (Figure 9-10). The mesenteric panniculitis did not show a significant restriction of diffusion on diffusion weighted images (DWI) and ADC map (Figure 11-12). Although, the mesenteric mass showed a delayed enhancement after injection of gadolinium due to its predominant or partial fibrotic nature, like reported in literature (Figure 13).

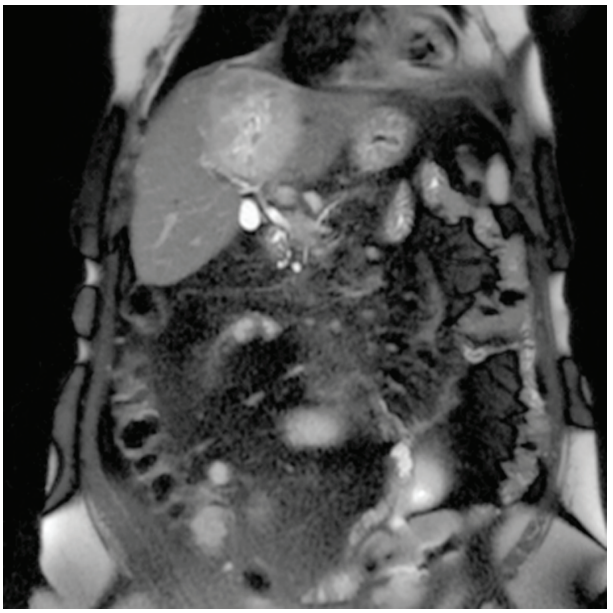

Figure 9. An axial T2 fat saturated image of abdomen shows the presence of a hyperintense mesenteric mass in the root of mesentery with a pseudocapsule around the mass

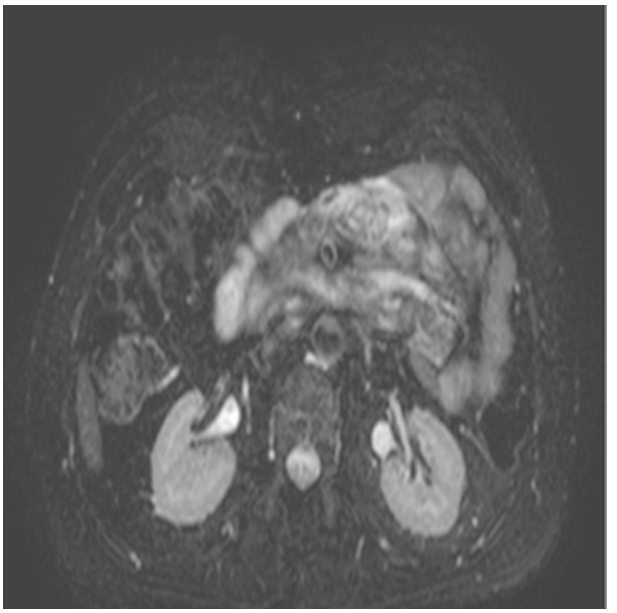

Figure 10. An axial T2 fat saturated image shows the presence of a large, hyperintense mesenteric panniculitis. The solid component is preponderant and the pseudocapsule and the fat ring sign are not clearly visible in this case

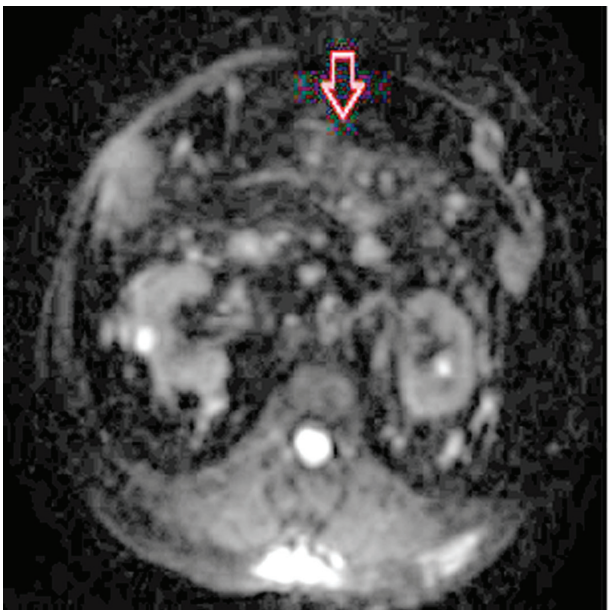

Figure 11. The mesenteric panniculitis does not show a restriction of diffusion on DWI image (red arrow)

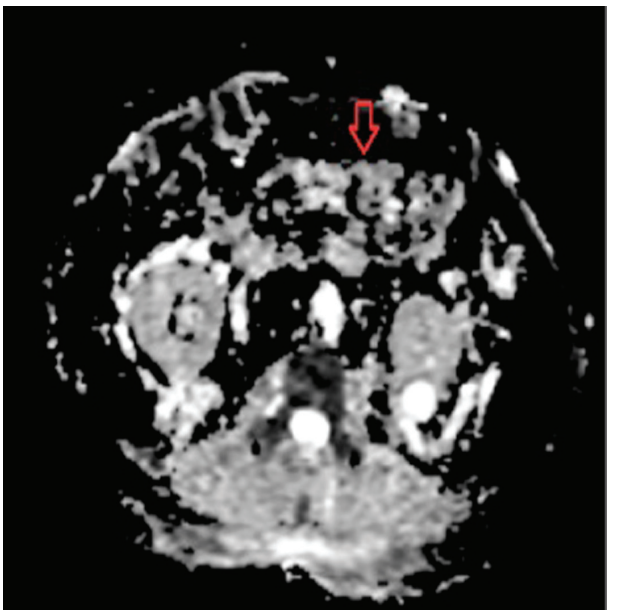

Figure 12. The ADC map in the same patient confirmed the absence of a significant restriction of diffusion (red arrows)

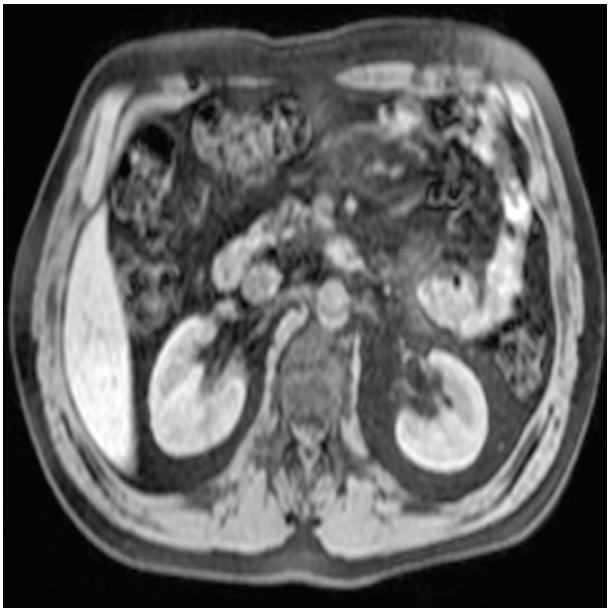

Figure 13. This axial contrast enhanced MRI image of abdomen during the late phase shows the presence of a mesenteric mass with contrast enhancement. The contrast enhancement is typically more evident in the late phase and more evident in the forms with a greater fibrotic component or in the fibrotic portions of the mass such as the pseudocapsula

Differential diagnosis

The differential diagnosis of mesenteric panniculitis includes various conditions. Mesenteric oedema, hemorrhage, lymphedema, inflammation and mesenteric neoplasia may mimick mesenteric panniculitis (37). The mesenteric oedema occurs in patients with hepatic cirrhosis, cardiac failure, renal failure, hypoproteinemia, bowel ischemia, venous thrombosis and primary or secondary tumors involving the mesentery or abdominal inflammatory disease such as pancreatitis, appendicitis and diverticulitis (37). The mesenteric oedema may be associated with subcutaneous fluid and ascites and, in these cases, the mesenteric oedema is usually related to a systemic disease. When the mesenteric oedema is associated with venous thrombosis, the oedema is usually focal and localized around the vessels occluded (42). However, it is interesting to note, that the conditions of acute inflammation could lead to chronic inflammation and chronic degeneration of the mesentery that could be considered a secondary form of mesenteric panniculitis.

The mesenteric hemorrhage is usually traumatic, iatrogenic or may be related with intraperitoneal tumor rupture. However, the differential diagnosis is not difficult because the mesenteric hemorrhage shows a density between 40-60HU and a high signal intensity in T1 weighted images.

Another disease which may mimick the mesenteric panniculitis is the peritoneal tuberculosis. The disease usually appears as a multiple nodularities of the mesenteric fat, thickening of the peritoneum with ascites and enlarged lymph nodes. However, the tuberculosis usually involves various part of the peritoneum like the omentum, spleen and liver, and the lymph nodes have a hypodense necrotic core, sometimes calcific (42, 43).

Among the tumors, the most common neoplastic process of mesentery is non Hogdkin lymphoma and 30-50% of non Hogdkin lymphoma harbouring in the mesenteric lymph nodes (44). The non Hogdkin lymphoma presents three main radiologic pattern due to coalescence of lymphomatous lymph nodes and/or mesenteric lymphomatous infiltration, which may mimick the mesenteric panniculitis: multiple, rounded, mildly enhancing, homogeneous masses that often encase the mesenteric vessels and produce the“sandwich sign”; a large heterogeneous mass with low-attenuation areas of necrosis; an ill-defined infiltration of the mesenteric fat with sometimes the fat ring sign around the vessels (37, 42, 44, 45). However, if multiple, large lymph nodes are visualizable with diffuse and irregular fat infiltration, which increase at imaging follow-up, the lymphoma must be the first hypothesis. In this context, the use of PET/CT is included in the differential diagnosis between mesenteric panniculitis and abdominal lymphoma with mesenteric involvement. In the study of Zissin et al., 33 PET/CT were evaluated in 19 oncological patients with mesenteric alterations morphologically due to mesenteric panniculitis and the uptake of mesenteric nodules was studied. In 11 patients, fluorodeoxyglucose uptake (FDG) was negative and remained negative at subsequent controls and therefore the authors concluded with certainty for a condition of benignity possibly due to a mesenteric panniculitis. The remaining 8 patients had a suspected FDG uptake and in 7 patients an underlying neoplasia was found (6 with abdominal lymphoma and 1 with

relapsing metastatic cervical tumor) (46). Along the same lines are Coulier B et al., according to which the absence of FDG uptake at PET/CT has a high accuracy in excluding a lymphomatous or carcinomatous neoplastic disease. In fact, the PET/CT is useful for the identification of mesenteric deposits or manifestations of neoplastic pathologies even in patients with a mesenteric panniculitis-like pathology and it is also useful for evaluating a possible subsequent onset of the disease. However, it must be considered that PET can be non-specific and an increase in uptake can be due to inflammatory forms such as sarcoidosis (47).

The appearance of carcinoid tumor and rectractile panniculitis can be identical. Both can appear as an ill-defined, infiltrating soft-tissue mass with calcification and desmoplastic reaction. However, the preservation of the fat around vessels and lymph nodes directs the diagnosis towards the mesenteric panniculitis. Although, the carcinoid tumor may be associate with a hypervascular bowel mass or hepatic metastasis (36).

The peritoneal carcinomatosis and peritoneal mesothelioma may simulate the sclerosing mesenteritis when soft tissue implants and lymphadenomegaly are localized in the mesentery. Calcifications may be present in both tumors. However, in these conditions we observe usually an extensive involvement of the peritoneum, with peritoneal thickening and ascites. These features are not typical for mesenteric panniculitis (36).

Conclusion

The mesenteric panniculitis is a rare disease of the mesentery. In most cases it is asymptomatic and its identification is occasional and occurs during the execution of diagnostic exams, performed for other reasons. When the disease is symptomatic, the symptoms are mainly abdominal pain and dyspepsia, however, in a small percentage it may produce intestinal obstruction and requiring surgery.

From a radiological point of view, the diagnosis of mesenteric panniculitis can be suspected whenever a mass is found in patients with abdominal symptomatology or not, in the root of the mesentery, characterized by dislocation of the surrounding structures, inflammation and variable fibrosis of the mesenteric fat, with a selective sparing of the fat around the vessels and the lymph nodes and with a fibrotic pseudocapsule around the lesion itself. This mass may also have a predominantly fibrotic and solid appearance and may determine traction or infiltration of the intestinal loops with their occlusion or perforation.

Therefore, it is important for the radiologist to know how to recognize this disease and evaluate its relationships with the surrounding structures.

Regarding the etiology, it is our opinion, endorsed by some experiences in literature, that mesenteric panniculitis may be secondary to various conditions causing chronic inflammation of the mesentery.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

What chance do we have to decrease prostate cancer overdiagnosis and overtreatment? A narrative review

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Summary. In the era of biochemical tests and algorithms, the management of prostate cancer from prevention to treatment is still controversial. The debate is focused on clinically-significant and clinically-insignificant prostate cancer. As it is well known, the diagnostic tools available are not able to distinguish between the two, thus leading to men treated for prostate cancer even if not strictly necessary. Unfortunately, as of today, there is no test available able to predict the clinical aggressiveness of prostate cancer at the time of the diagnosis. However, some indexes, PSA derivatives, immunocomplexes, and diagnostic methods have been proposed. If properly used in the daily clinical practice, these tools may be of support in the decision making process, in the effort to reduce the overdiagnosis and the overtreatment of prostate cancer.For this reason, we believe that a clear knowledge of this tools, indexes and diagnostic methods is of the utmost importance in preventing the morbidities related to unnecessary treatment as weel as preventing the detrimental effect of missing the diagnosis of a clinically significant prostate cancer.This reviews encompasses the most studied tests and diagnostic methods to predict the aggressiveness of prostate cancer, to avoid to miss a diagnosis of clinically significant cancers and to optimize the overall pre-treatment work-up. (www.actabiomedica.it)

Key words: prostate cancer, PSA-IgM, iXip, PCA3, PHI Index, Targeted biopsy

Introduction

The advent of PSA testing more than two decades ago has improved the early detection of prostate cancer, leading to more men being diagnosed and treated.

Interestingly, it is still controversial whether the increased detection and treatment of prostate cancer has led to increased overall survival rates. Data from two long-term screening studies were published in the last few years and reported conflicting results. The Prostate, Lung, Colorectal and Ovarian (PLCO) screening concluded that there is no difference between men who were screened and men who were not (1). On the other hand, the European Randomized Study of Screening for Prostate Cancer found a 20% reduction in the mortality rate in screened men (2).

Actually, many studies have tired to characterize the extent of overdiagnosiss and overtreatment of prostate cancer resulting from prostate cancer screening, with higly variable results. A review of the major studies on overdiagnosis and overtreatment of clinically localized prostate cancer has been published by Loeb S et al (3). According with this review, prostate cancer overdiagnosis ranges from 1.7% to 67%. There are many reasons of such a disparity in the results of the sudies included in this review, all related to the time period of the studies and the features of the undelying populations (e.g. age, comorbities). The definition of overdiagnosis plays a role, also.

However, when a diagnosis of prostate cancer has been made, the major issue is the following decision on treatment, ranging from active surveillance to radi-

cal surgery. The lesson learned from the results of active surveillance protocols shows that not all prostate cancers require active treatment, as not all are life-threatening.

In his editorial on prostate cancer overdiagnosis, Roobol MJ and Schroder F highlight that unfortunetely, as of today, there is no test or combination of test available that can give a yes-or-no answer to the risk of having a life-threatening prostate cancer (4).

Even if there is not a test able to predict if the treatment of a prostate cancer would result in an overtreatment, there are still screening methods, algorythms and diagnostic pathways able to be of help.

Methods of screening for prostate cancer

The European Randomized Study of Screening for Prostate Cancer (ERSPC), with a 30 years follow-up demonstrated that the number needed to treat is decreasing as well as the number needed to screen (6). The results of this study are reported in table 1.

The screening for prostate cancer may be systematic or opportunistic. A comparison of systematic an opportunistic screening suggested overdiagnosis and mortality reduction in the systematic screening group compared to a higher overdiagnosis with a marginal survival benefit in the opportunistic screening regimen (5). Similar results were found in a Cochrane review update (7), indicating that similar to breast and cervical cancer screening, organized screening is more effective than opportunistic in reducing disease-specific mortality.

As Arnsud Godtman R et al report, there are many reasons why opportunistic screening is less effective in achieving the aim of a recuction of mortality, including inappropriate screening density (8) or inappropriate follow-up after a first positive test, screening a people who do not belong to a group of patients who may benefit from screening, due to comorbilities or age.

PSA-IgM and iXiP

It is well known that in healthy persons immunoglobulins are expresseed only on the surface of B-lymphocytes. However, contradictory to this theory, almost all the subclasses of immunoglobulins have been found to be expressed by malignant cells of epithelial origin (9).

In more details, Immunoglobulins M (IgM) are abnormally espressed in liver (10), prostate (11), ovarian (12) and laryngeal (13) cancer. Serological levels of the immunocomplex PSA-IgM is reported to be accurate for the early diagnosis of prostate cancer and have been included in an algorithm to define the iXip, an index able to determine the probability for having prostate cancer (11,14). The output generated by the algorithm is a numerical value ranging from 0 to 100% and directly correlates to the risk of diagnosing a prostate cancer at biopsy.

The algorithm generating the iXip index is based on the value of PSA, the immunocomplex PSA-IgM, prostate volume and patient age. This index was initially created to improve the diagnostic performance of PSA, however it showed to be able to reduce the number of repeat biopsy in patients with a previous negative biopsy and still under suspicion for prostate cancer (11).

The PROXIMA study is a promising ongoing trial. It is a prospective trial whose aim is to demonstrate the ability of iXip to predict the presence of a clinically significant prostate cancer, defined as prostate cancer with a Gleason score > 6.

Prostate Cancer Antigen 3 (PCA3)

The Prostate Cancer Antigen 3 (PCA3) gene, formerly known as DD3, was first identified in 1999 (15). It is non-coding mRNA highly expressed in prostate cancer tissue. In 2003, PC3 mRNA levels showed to be strongly associated with prostate cancer, leading to the development of a urinary assay able to measure this analyte (16).

The PCA3 test is intended for reducing unnecessary biopsies, while maintaining or increasing the detection of prostate cancer.

Kusida Y et al. investigated the expression on PCA3 in lymph node micrometastases. Among the patients with biochemical recurrence, a vast majority showed to be positive to PSA and/or PCA3 if investigated for micrometastases. As lymph node involvement may is known to be an indicator of poor clinical outcome in patients diagnosed with prostate cancer, PCA3 may be supposed to be able to play a role in the identification of clinically significant prostate cancer (17).

However, to our best knowledge, only a few studies addressing PCA3 as a predictor of clinically-significat tumor report data on a long-term period (17,18). In all these study, PCA3 did not achieve the requirements for validation as a marker for intermediate or surrogate outcomes.

Pro-PSA and [-2]pro-PSA

Pro-PSA is a precursor of PSA. One of its isoforms, the [-2]pro-PSA is the more stable form of PSA. It is expressed in the peripheral zone of the prostate and is reported tho have higher levels in the serum of patients diagnosed with prostate cancer (24).

This marker may be considered in patients with high level of PSA in the intent to avoid them to undergo unnecessary biopsies.

In order to improve the performance of PSA and p2PSA to detect prostate cancer and to reduce the number of patients diagnosed with clinically-unsignificant prostate cancer, two other derivatives od p2PSA have been proposed. They are the percentage of p2PSA (%p2PSA) and the Beckman Coulter Prostate Health Index (PHI) (25,26). Compared to the Gleason score, %p2PSA has a sensitivity of 96% and a specificity of 9% for detecting aggressive disease while PHI has a sensitivity of 90% and a specificity of 17% (26).

Multiparametric Magnetic Resonance (mpMR) and targeted biopsy

Targeted biopsy of the prostate following Multiparametric Magnetic Resonance (mpRM) is an alternative to standard transrectal ultrasonography-guided biopsy (TRUS-GB) for prostate cancer detection.

Multiparametric Magnetic Resonance has gained interest in the last few years for its ability in visualising lesions within the prostate, due to its superiority in soft tissue resolution with anatomical zonal delineation that makes it useful in distinguishing indolent from aggressive disease (19). The PROMIS and PRECISION studies confirmed the superiority of mpRM and MR-targeted biopsy to TRUSGB. Also, these studies report the superiority of targeted biopsy in diagnosing clinically-significant prostate cancers (20-21).

The more recent study of van der Leest M et al confirms the "no immediate biopsy approach" after non-suspicious mpMR scans. The MR-pathway compared with the TRUSGB pathway results in an identical detection rate of clinically-significant prostate cancer, with significantly fewer non clinically-significant prostate cancer cases (22).

Conclusion

Despite high prevalence of disease, most prostate tumors are indolent and are unlikely to progress to clinical significance. In addition, every kind of prostate cancer treatment, as well as active surveillance, may lead to comorbidity and complications.

Another crucial point in prostate cancer diagnosis is the prostate biopsy: although it is generally well tolerated, prostate biopsy is an invasive diagnostic tool and is reported to carry side effects and complications.

This study focuses on the methods to reduce overdiagnosis and overtreatment of prostate cancer and may shed more light on the tests and diagnostic tools available.

As a consequence, the proper usage of tests, tools and algorithms able to detect clinically significant prostate tumors may be of help when candidating a patient to treatment, thus achieving the goal of reducing the impact of surgery, radiotherapy, chemoteraphy and other strategy of treatment.

In other words, keeping into account the additional information carried by these tests could give the opportunity to make a more informed, scientific decision with regard to choosing optimal candidates to a specific therapeutic strategy.

Even if an algorithm or an index able to integrate the information given by all these tests and tools has not been reported so far, it is likely that in the next future a comprehensive index for prediction of clinically-significance or aggressiveness of prostate cancer will be available, as already proposed for candidating patients to specific prostate cancer treatments, such as High-Intensity Focused Ultrasound (23) or other therapeutic strategies.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

CT-guided biopsy of subdiaphragmatic small renal nodule with the coaxial technique using MPR images

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Summary. *Purpose:* To demonstrate the advantages of CT-guided biopsy of subdiaphragmatic small renal nodule with the coaxial technique using MPR images. *Methods:* The study included retrospectively 12 patients who underwent CT-guided biopsy with 18 G needle of subdiaphragmatic small renal nodule (<15 mm) suspected. Histology reports have been the reference standard. *Results:* The histology was diagnostic in 12 biopsies (100%): out of 10 neoplastic nodule (83.3%), 8 were malignant (80%) and 2 were benign (20%); 2 out of 12 were non-neoplastic (16.6%). No procedural complications were observed (0%). *Conclusions:* By using MPR images there is an effective improvement during coaxial CT-guided biopsy of subdiaphragmatic small renal nodule difficult to reach and to reduce the complication as pneumothorax. (www.actabiomedica.it)

Key words: small renal nodule, CT-guided biopsy, MPR images, coaxial technique

Introduction

Diagnostic imaging is very important in the detection of tumors in the histological characterization through imaging-guided biopsies (1, 2).

At present, percutaneous CT-guided Biopsy is a highly diagnostic and minimally invasive procedure, and it is often possible to avoid removing tissue to be examined surgically thanks to the support of imaging (3, 4).

The Multiplanar Reconstructions (MPR) technique during CT-Guided Biopsy, can significantly increase the number of lesions which can be biopsied percutaneously thus reducing the need of surgical techniques (2, 5-6).

MPR images are helpful in the pre-operative assessment of the most useful course of the needles that are used for biopsies of thoracic, abdominal and retroperitoneal lesions (7-10).

The renal lesion located at the subdiaphragmatic space is difficult to reach with a traditional coaxial Ct-guided biopsy, without having a pass through the lung, with the risk of pneumothorax.

Both ultrasound (US) and multi-detector computed tomography (MDCT) are used as guide for biopsy procedures; while US allows the real-time evaluation of the course of the needle and is not associated to ionizing radiations (11-14), MDCT is helpful in the detection of small lesions that are located in anatomic sites difficult to evaluate with US (15-16).

There is no study that analyzes the positive contribution of MPR images to CT-guided biopsy for small renal lesion (<15 mm) difficult to reach, in particular renal lesion located into the subdiaphragmatic space.

The aim of this study was to demonstrate the advantages of CT-guided biopsy of subdiaphragmatic small renal nodule with the coaxial technique using MPR images, which are impossible to reach with conventional non-surgical biopsy techniques.

Materials and methods

Patient population

We retrospectively included in our study 12 patients (7 males, 5 females; age ranging from 52 to 68 years) that underwent CT-guided biopsy for small renal lesions (size of the lesion between 9 and 14.8 mm) in the period from May 2017 to February 2018. The indication to the CT-guided biopsy was represented by a nodule that could not be biopsied through ultrasound or axial CT scans, for his difficult position.

We have excluded patients with a known tumor history, pediatric patients and those with bleeding diathesis.

CT-guided Biopsy Technique

The patients' informed consent was obtained in all cases.

The procedures were all performed with a 64-detector helical scanner (Optima CT540, GE Healthcare, USA), without the administration of contrast media; the following technical parameters were used: 100 kV, 100 mAs, 2 mm collimation, 2.5 slice thickness with 1 mm of reconstruction increment. The multi-planar reformatted images were obtained through a dedicated software of a post-processing workstation (AW Server, GE Healthcare, USA). All patients were biopsied with a coaxial technique, using MPR images (Fig. 1).

The extemporaneous microscopic evaluation of the biopsy's samples to be analyzed has been evaluated by our laboratory.

One experienced radiologist with 7 years of experience in CT-guided biopsies performed the procedures.

The patients were placed on the CT bed in various positions depending on the site of the lesion that should be biopsied.

The first step was the acquisition of the axial scans, followed by the multi-planar reconstruction in order to decide the most adequate trajectory of the 14-Gauge needle (Cook Medical, Bloomington, USA).

Once the stylet of the guide was removed, a second needle was introduced, namely a 16-Gauge needle.

A second CT scan and the respective MPR images allowed evaluating if the needle was in the correct position and if position was adequate the biopsy sample was taken.

To exclude any procedure-related complication, a third scan was obtained after the needle was removed; the patients were kept under clinical observation for 4 hours after the biopsy in order to exclude major complications.

The histologic report was used as reference standard for assessing the accuracy of the procedure.

Statistical Analysis

The overall diagnostic accuracy of CT-guided biopsy of renal small lesions was calculated as: true posi-

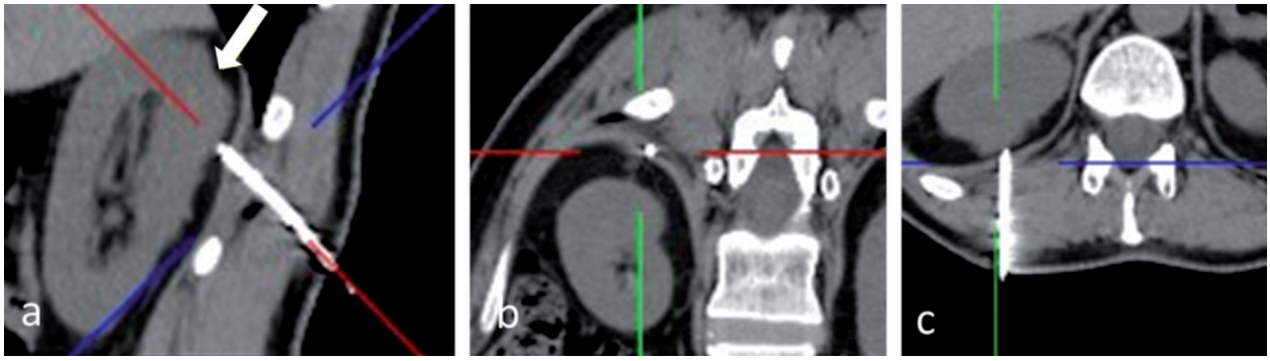

Figure 1. a-c. 62 years old male with renal nodule (arrow) located at the upper lobe, very difficult to achieve with a traditional coaxial biopsy, without having to pass through the lung with the risk of having a pneumothorax. CT-guided biopsy in three different scanning planes, with coaxial technique with MPR images (a-c), with needle-track in the subdiaphragmatic space

tive (TP) + true negative (TN)/TP+TN+ false positive (FP) + false negative (FN) (17).

Results

Twelve CT-guided biopsy with coaxial technique and MPR images were performed (Table 1) (Fig. 1, 2).

All the target lesions subjected to biopsy were all located at the upper renal level.

In all cases, the CT-guided biopsy technique coaxial with MPR images enabled us to detect the tip of the needle within the target lesion (100%).

The biological material was considered to be suitable for biopsy study, with a diagnostic value, in 12 biopsies (100%); we did not have inadequate sample.

Among these, 10 out of 12 (83.3%) were found to be neoplasms (Table 1), of which:

- 8 were malignant primary renal lesions (66.6%);
- 2 were oncocytoma (16.6%);

Two out 12 (16.6%) were Angiomyolipoma (Table 1).

The overall diagnostic accuracy of the method was 100%.

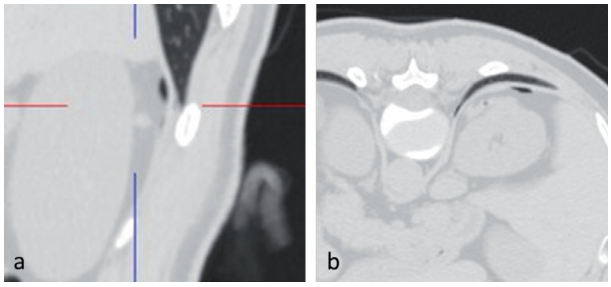

Figure 3. Control after Ct-guided biopsy: the needle track has been subdiaphragmatic without the onset of a pneumothorax

Procedural complications did not occur in any of the 12 cases (0%) (Fig. 3).

Discussion

The MPR images allowed collecting biological samples useful for a diagnosis through CT-guided biopsies with coaxial technique in 100% of cases (Figs. 1, 2); the diagnosis of renal small lesions was obtained in 100% of the cases, without the need to perform a surgical biopsy.

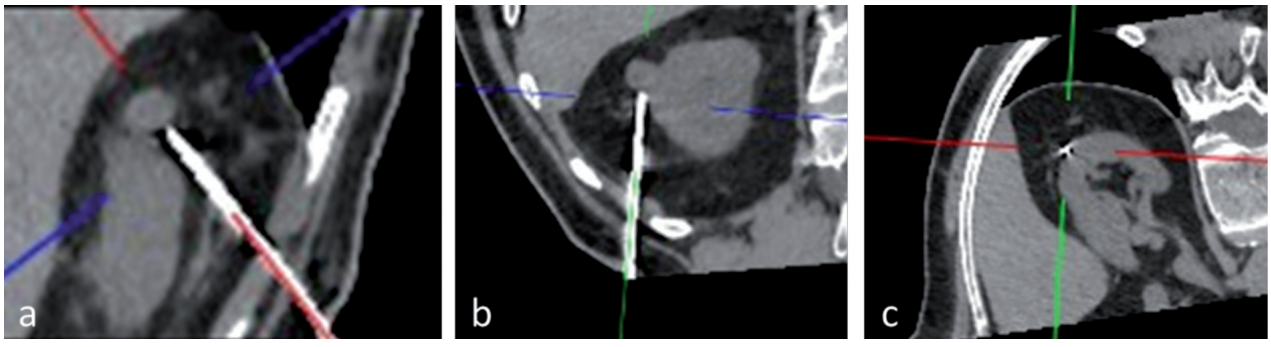

Figure 2. a-c. 57 years old male with renal nodule located at the upper lobe. CT-guided biopsy in three different scanning planes, with coaxial technique with MPR images (a-c), with needle-track in the subdiaphragmatic space

Table 1. Bioptical diagnosis after CT-guided coaxial biopsy with MPR technique (n: number of cases)

	n (%)		n (%)
Renal Tumor	10 (83.3)	Renal Cell Carcinoma	8 (66.6)
		Oncocytoma	2 (16.6)
Angiomyolipoma	2 (16.6)		

In case of small renal lesions as our population, unreachable through US, CT-guided biopsy represents the best option to obtain tissue samples (2, 15-16); moreover by using reformatted images it is possible to biopsy lesions that cannot be reached with axial CT images alone (7-10).

Our study population presented renal small lesions (<15 mm) suspected of malignancy in previous radiological imaging; all the nodules were located at the subdiaphragmatic level, very difficult to achieve with a traditional coaxial biopsy, without having to pass through the lung with the risk of having a pneumothorax (Fig. 3) (17-20).

With the MPR images we have found the best and safest needle trajectory (6, 18-21).

To our knowledge, studies concerning the effectiveness of the coaxial technique using MPR images for CT-guided biopsies for small renal lesion, located in a difficult area like the subdiaphragmatic region, have not yet been carried out.

Our study suggests that the advancement of CT-guided coaxial biopsy technique in small renal lesions can be successfully performed thanks to this addiction to MPR methods, having the possibility to reduce the complication, in particularly the pneumothorax, and to find the best needle trajectory.

Our study presents a certain amount of limitations; the study was retrospectively and the number of cases was small.

This is explained by the exclusion of all those lesions in which it was possible to perform a biopsy with simple coaxial technique without using MPR reconstructions, such as the lesion located at the mid and lower renal pole and with the exclusion of lesions above 15 mm.

In conclusion by using MPR images there is an effective improvement during coaxial CT-guided biopsy of subdiaphragmatic small renal nodule difficult to reach and to reduce the complication, as pneumothorax.

The paper was performed as the Declaration of Helsinki: ethical principles for medical research involving human subjects.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Residual interfragmentary gap after intramedullary nailing of fragility fractures of the humeral diaphysis: short and midterm term results

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Summary. The purpose of this retrospective study was to evaluate the short and midterm radiological outcome with regards to bone healing, correlated with humeral shaft fractures treated with anterograde intramedullary nailing, when interfragmentary gap was significant. Here, we critically review our experience of short and midterm term results in over 65 year-old patients. Inclusion criteria in the study were: (1) patients over 65 years old (2) patients with displaced humeral mid shaft fractures (AO/OTA 12 A B C type fractures); (3) patients treated with closed reduction and internal fixation with intramedullary nail (Trigen Humeral Nail® Smith & Nephew) with at least two screws for proximal locking and one screw for distal locking; (4) residual interfragmentary gap, being considered significant any gap >6 mm (being 7 mm the minimum nail diameter available in our facility). Various factors were considered in our analysis: the size of the interfragmentary gap in both projections (also the mean of the measured gaps was calculated), the relationship between the greater and the mean interfragmentary gap and the second diameter measurement of the nail in the half distal part, the number of the screws in distal locking procedure, the use of a reaming procedure or not, the AO classification, the actual age at the time of surgery, the operating time, the nail second diameter as described before and its ratio with the measured residual gap. At 3 months follow-up, 4 patients showed radiographic healing (26,67%), 9 patients showed a visible callus (60%), with a total of 13 patients (86,67%) showing signs of normal recovery, the remaining 2 patient had insufficient callus formation (13,3%). At 6 months follow-up, 1 patient was missing (6,67%), although radiographic healing was already evident during the previous follow-up check, another one showed incomplete callus formation, the remaining 13 patients showed radiographic healing (86,67%), with a total of 14 patient considered healed at 6 months follow-up (93,33%). In conclusion, osteosynthesis with anterograde nail in geriatric patients appears to be a quite safe approach despite a great interfragmentary gap. After 6 months of treatment, callus formation and the overall clinical outcome were proven to be above satisfaction. (www.actabiomedica.it)

Key words: interlocking nail, humeral shaft fractures, interfragmentary gap

Introduction

There is no consensus among physicians on the best approach to treat humeral shaft fractures in the elderly; at present, all proposed treatments are recommended, but a gold standard method has not been developed yet. Several non-surgical procedures such as functional bracing, as well as surgical methods like anterograde and retrograde intramedullary nailing, plating or external fixation are frequently used for the treatment of these fractures (1). Nevertheless, none of these approaches is complication-free; in fact, many

technical circumstances like comminution, open fracture and unstable fixation can woefully lead to a fixation failure. Ultimately, in geriatric patients, the goal of treatment is a quick comeback to the daily activities.

Several recent studies report that the treatment of humerus shaft fractures with locked anterograde intramedullary nailing provides enough fixation and possibility of early movement of the shoulder and elbow joints. This approach is typically coupled with satisfactory radiological and functional results (2). It is broadly claimed that humeral nailing should provide a kind of fixation that restores length, alignment, and rotation of the humeral shaft fractures. Nevertheless, a real functional reduction by nailing is usually demanding and it is very common that a residual fracture gap, due to distraction in the fracture site, does not allow for a satisfactory radiological outcome. Unfortunately, many orthopedic surgeons deal with intramedullary humeral nailing without focusing to anatomic reduction of the fragments, an event that usually affects functional recovery.

The aim of this retrospective study was to evaluate the short and midterm radiological outcomes, with regards to bone healing, in humeral shaft fractures treated with anterograde intramedullary nailing, in the presence of an important interfragmentary gap. We therefore report our experience as well as the short and midterm results in over 65-year-old patients.

Patients and Methods

We retrospectively evaluated the outcomes in patients with a fragility fracture of the humeral shaft, treated at San Carlo Borromeo Hospital (Milan, Italy) from November 2012 until May 2017. An informed consent regarding the use of clinical data for scientific publication was obtained. The humeral shaft fractures in this study were identified according to the AO classification. According to the National Osteoporosis Foundation, any fracture resulting from a fall from a standing height or less is considered a fragility fracture. This is typically associated with low bone mineral density or established osteoporosis, and it is common in elderly patients.

The patient inclusion criteria in this study were the following:

(1) age ≥65 years (2) diagnosis of displaced humeral mid shaft fractures (AO/OTA 12 A B C type fractures); (3) patients treated with closed reduction and internal fixation with intramedullary nail (Trigen Humeral Nail® Smith & Nephew), with at least two screws for proximal locking and one screw for distal locking;

to note, this type of nail (Trigen Humeral Nail® Smith & Nephew) present a difference in diameter between the proximal (epiphyseal) section and the distal (diaphyseal) one, the second being smaller than the former: in the present article, any reference to a nail diameter is meant to be referred to the distal diameter. (4) presence of a residual interfragmentary gap > 6 mm of diameter (being 7 mm the minimum distal nail diameter measurement available in our facility).

The patient exclusion criteria were the following:

(1) Comorbidity Severity Score (ASA) ≥4; (2) a post-operative follow-up shorter than 6 months; (3) the presence of articular fractures or fractures that involved the humeral head; (4) open fractures; (5) isolated transverse fractures of humeral shaft (AO/OTA A3).

X-ray images of all the eligible patients were obtained from the Picture Archiving and Communication System (PACS) sited in our Institute. The images were obtained at three different views (anteroposterior, intra or extra and rotation views) immediately after osteosynthesis, and in at least two standard projections (anteroposterior and lateral views) at follow-up.

The surgical technique consisted of a synthesis with an anterograde Trigen nail (S&N). All patients were placed in supine position on a radiolucent table. Cephazoline (2 g) was administered 30 minutes prior to skin incision for infection prophylaxis. A proximal anterolateral trans-deltoid surgical access was used: a small skin incision was performed from the anterolateral edge of the acromion distally towards the deltoid insertion; subsequently, the deltoid muscle fibers were retracted, and the supraspinatus tendon was longitudinally incised for 1 cm and sutured at the end of the procedure to minimize the damage of the rotator cuff. The nail's entry point was medial to the greater tuberosity of the proximal humerus. After the insertion of a guide wire through the opening made in the supraspinatus tendon, the surgeon proceeded to indirect reduction of the fracture.

Multiple controls under image intensifier were necessary. Several nail diameters were used. Two or three screws were used for proximal locking. A single or double screw distal locking was performed with the Sureshot System (S&N®). A single arm sling was positioned to all patients lasting for two weeks after surgery. Assisted mobilization of the shoulder was recommended to each patient.

All patients were followed up as outpatients with X-ray control scans according to our protocol (AP, intra, extra and rotation projection of the humerus) after 40 days, 3 months, and 6 months, and thereafter at regular intervals depending on the progress of fracture healing. After each follow-up, proof of the clinical examination was obtained. When available, clinical data were also recorded through the hospital database.

Bone union and complications such as pseudarthrosis, infections and nerve lesions were evaluated in the immediate post-operative period. In addition, correct function of the rotator cuff tear was also evaluated.

Bone healing and relative complications, such as the presence of a radiopaque callus in the interfragmentary gap, were recorded in every patient in at least one of the three projections at 3 and 6 months of follow-up.

The bone callus already formed at the fracture site was observed at 3 and 6 months of follow-up and compared to the previous postoperative x-rays. A single scale for measure of callus formation was used (insufficient, visible and advanced) and clinical data were simplified for statistical use (very limited function, limited function, good function, excellent function). Several factors were considered in our analysis: the size of the interfragmentary gap in both projections (the mean of the measured gaps was also calculated), the relationship between both the maximum and the mean interfragmentary gap values and the nail diameter, the number of screws used during the distal locking procedure, the use of a reaming procedure or not, the AO classification, the patient's age at the time of surgery, the surgical procedure length, the nail diameter and its ratio with the measured residual gap.

The radiological and clinical outcomes, as well as the presence or absence of complications in the short and midterm periods were evaluated in relationship with the aforementioned factors.

Results

The cohort of our patients that met the inclusion criteria was composed of 22 subjects. There were 17 female and 5 male patients with a mean age of 76,2 (± 7) years (range: 65-88 years). The AO classification was used to categorize the fractures (Table 1): A1, 8 cases; B1, 6 cases; B2, 4 cases; B3, 2 cases; C1, 2 cases.

The mean surgery time was 88 (± 25,7) minutes (range: 50 - 140 min).

Two or three proximal screws were used for proximal locking (13 patients with 2 screws, 9 patients with 3 screws); single or double-screw distal locking was performed with the Sureshot System (S&N®): 15 patients receiving one screw, 7 patients receiving two screws. The diameter of the nail used was 7 mm in 16 patients, 7.5 and 8.5 mm in 3 patients for each nail diameter, respectively.

A reaming technique was performed in 13 patients (59,1%), while the remaining 9 were treated with a non-reaming technique. No intra-operative or other major complications were observed, like non-unions, nerve lesions or infection. We reported only

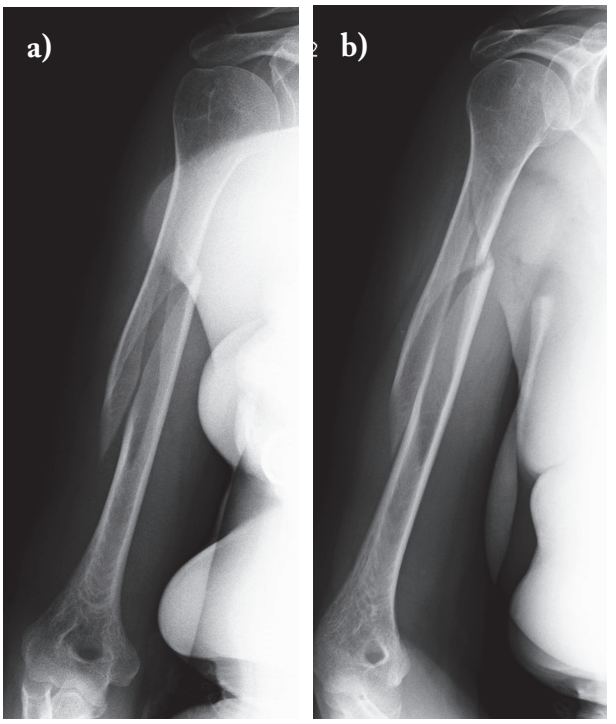


Figure 1 a, b. Female patient, 78 years old, 12B1 type humeral fracture according to the AO/OTA classification

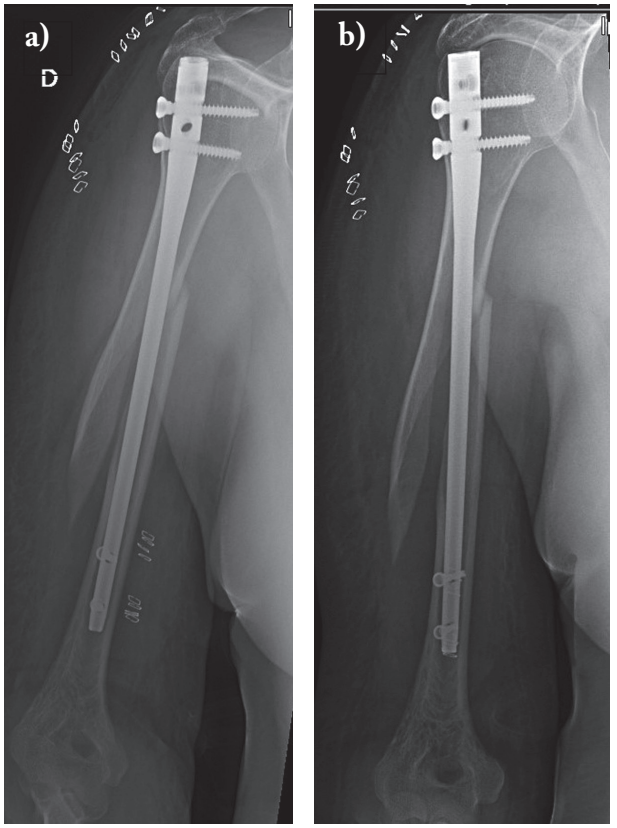


Figure 2 a, b. Post-operative images, a 7 mm diameter of Trigen S&N nail was used with 2 distal locking screws and 2 proximal screws. Interfragmentary gap is 1,46 cm in figure 2a and 1,7 cm in figure 2b

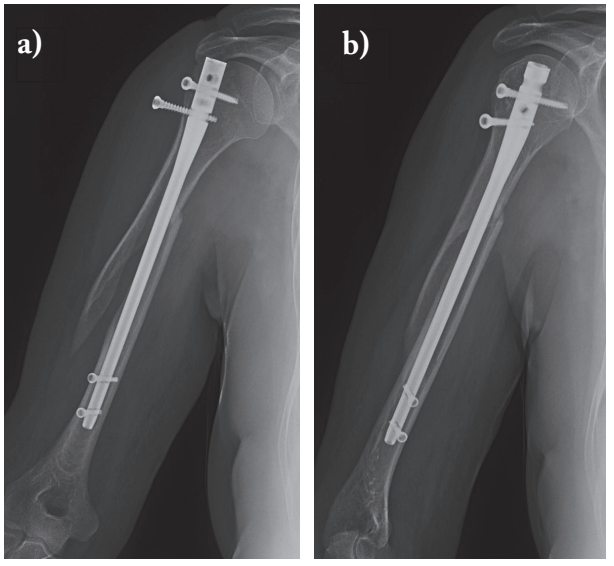


Figure 3 a, b. X-rays one month after surgery. No signs of bone callus. Mobilization of one of the proximal screws

a complication that could be related to the surgical procedure: a patient reported a rotator cuff tear documented by MRI three months after surgery. In another patient, the proximal screw was removed after one month because of screw loosening. No other complications were observed. Regarding residual post-reduction gaps, the mean value of the intra-rotation gap was 0,97 (± 0,53) mm, the mean extra-rotation gap was 0,89 (± 55) mm, and the overall mean gap was 0,93 (± 49) mm, the maximum mean gap measured in both projections was 1,07 (± 0,49) mm. The ratio between gap and nail diameter values was also measured, being 1,27 (± 0,67) when considering the mean gap value, and 1,47 (± 0,67) when considering the maximum gap value.

Seven patients did not present themselves at follow-up; hence, the radiographic results are related to only 15 patients (*n*=15). The X-ray images obtained at 6 months of follow-up were missing for one of them. However, the patient was not excluded since the 3 month-x-rays showed complete healing.

At 3 months of follow-up, 4 patients showed radiographic healing (26,67%), 9 patients showed a visible callus (60%), 2 patients showed insufficient callus formation (13,3 %). Overall, a total of 13 patients showed radiographic signs of recovery (86,67%).

At 6 months of follow-up, 1 patient's images were missing although radiographic healing was already evident during the previous follow-up check, 1 patient showed incomplete callus formation (6,67%), the remaining 13 patients showed radiographic healing (86,67%), with a total of 14 patient considered healed radiographically at 6 months of follow-up (93,33%).

Regarding clinical data, at 3 months of follow-up, 1 patient showed very limited function (6,67%), limited function was seen in 7 cases (46,67%), good function in 5 patients (33,33%), excellent function in 1 patient, while data for the remaining patient were missing; therefore, a total of 40 % showed clinical acceptable results.

At 6 months, clinical data from 2 patients (13,3%) were missing, function was reported as very limited in 1 patient (6,67 %), limited in 1 patient, excellent in 2 patients (13,3%) and good in 7 patients (46,67%), with a total of 11 patients (73,3%) showing acceptable clinical results.

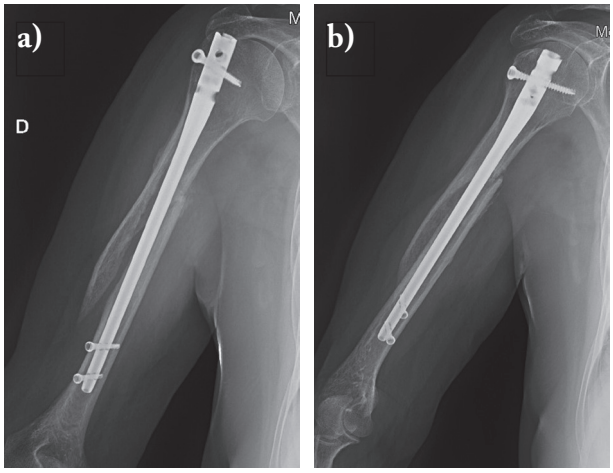


Figure 4 a, b. X-rays 3 months after surgery: despite of the initial gap, bone callus is already visible. The mobilized screw has been removed

Statistical analysis was performed to identify factors able to influence clinical prognosis. When applicable, the chi-square test was used to compare clinical and radiographic results, as well as the presence or absence of complications, analyzing categorical variables (AO classification, nail diameter, number of screws, reaming). If the chi-square requirements were not met, Fisher exact test was used, by converting the outcome index in nominal variables (acceptable vs not acceptable result). Age and length of surgery were compared by a Kruskal-Wallis test to study the effect of the reduction gap in the outcomes.

None of the categorical factors considered was correlated with a difference in x-ray or clinical follow-up at 3 months. Only one patient did not radiographically show complete healing after 6 months, therefore no statistical analysis could be performed in that case. Similarly, clinical results at 3 and 6 months were not influenced by any one of the factors considered; also, no factor was detected as related to the onset of complications.

Analyzing parameters such as age, length of surgery and gap measurement, no statistically significant differences were found in terms of clinical outcome at 3 and 6 months of follow-up. Moreover, there were no significant differences in age and length of surgery in the various outcome groups at radiographical follow-up at the same time points. Only the quality of reduction, namely the gap measurement, showed a significant difference at 3 months of radiographic follow-up between patients with different outcomes: patients with less callus formation showed significantly higher gap as the median of the gaps was 0,5 cm in patients with advanced callus formation versus 1,89 cm in patients with low callus formation (p<0,005), as shown in Graph 1. Remarkably this difference disappeared at 6 months of follow-up, since almost all patients were healed. To note, this difference was significant only

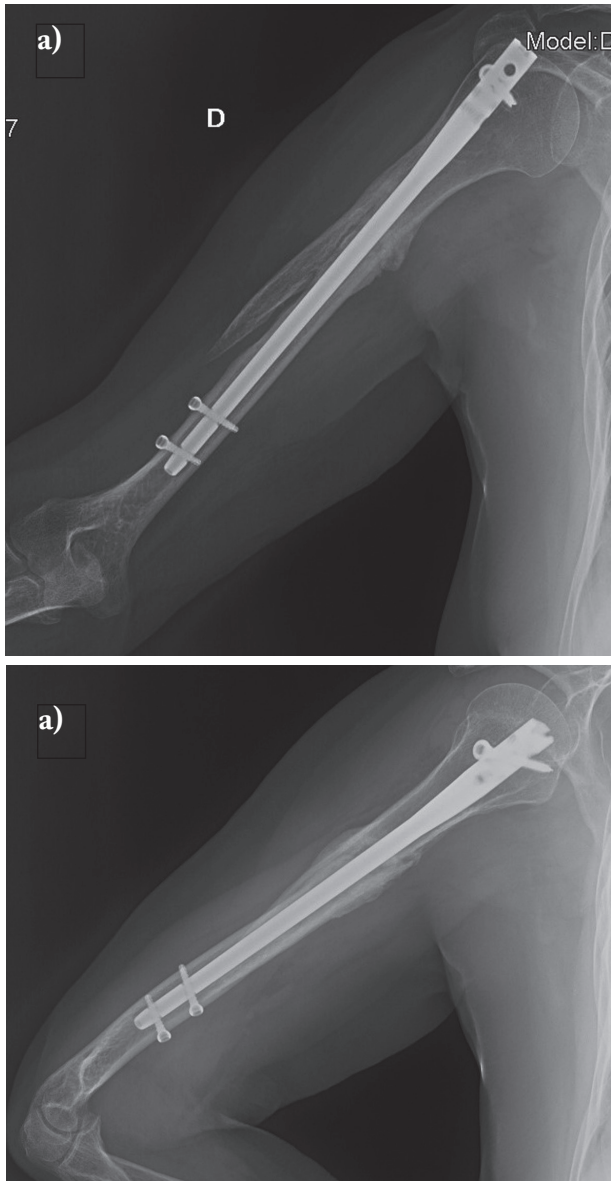
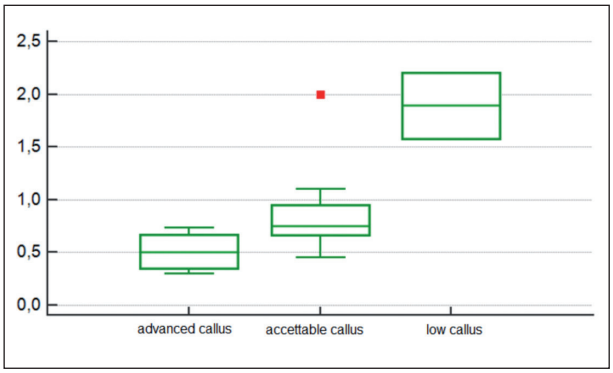


Figure 5 a, b. X-rays 6 months after surgery: the bone callus is visible with clear signs of fracture healing



Graph 1. The following graph represents interfragmentary gap values (Y axis) related to callus formation at 3 months follow-up (X axis). The red dot is an outlier. The significantly lower callus formation Is clearly shown in patients with bigger post-operative gaps

when considering the mean gap value, and not using the maximum gap value. Moreover, no differences in the influence on the outcomes were shown when calculating the gap as a ratio with nail diameter, being only the 3-month radiographic follow-up significantly influenced by the variable.

Discussion and Conclusion

Despite functional brace treatment being the gold standard in the conservative treatment of humerus shaft fractures, intramedullary nailing is the most common surgical method of fixation. Surgical treatment significantly decreases the risk of bad union and non-union, which are the most important complications of the conservative treatment (3).

Many authors consider plate fixation with open reduction or the Minimally Invasive Plate Osteosynthesis (MIPO) technique as the most appropriate methods of surgical treatment, but they are technically more demanding (4, 5). Compared to these two approaches, the intramedullary nailing is a faster procedure and could be more applicable in geriatric patients. Intramedullary fixation has the advantage to act as a load-sharing device and to preserve the periosteal blood supply around the fracture, so as to allow healing.

Decreased rotator cuff function after anterograde nailing of the humerus has been thought to be the cause of painful and decreased motion of the shoulder joint in active patients, providing an overall unsatisfactory functional result. The surgical technique has been cited as the main reason for rotator cuff dysfunction in young patients; however, in over 65-year old patients, if a proper surgical technique is practiced, the risk of rotator cuff tear is reduced, and satisfactory functional outcome scores can be expected in the post-operative period after intramedullary nailing for humeral shaft fractures (6).

In our study, we did not make a comparative study, for example between intramedullary nail and locking compression plate, as our findings (93,33 % of radiological healing and 73,3 % of acceptable clinical results at 6 months follow-up) were more than satisfactory.

Rommens et al. recommend avoiding diastases in the fracture gap, closing it by manual compression or through a compression device before static interlocking (7). Watson and Sanders claim that, after nail placement, there is often a distraction of the construct; according to them, this often leads to a non-union (8). Baltov et al. found a significant positive correlation between humeral shaft fractures locked in distraction and the risk of non-union. In fact, in their retrospective study they evaluated 111 patients with humeral shaft fracture treated with an intramedullary nail. They reported fracture distraction in 12,6% of the cases, in some of them greater than 4 mm (4,5%). Also, 2,7% of their patients showed delayed healing and 1,8% presented non-union (9).

In our retrospective study, we evaluated only geriatric patients with an interfragmentary gap >6 mm.

After intramedullary nailing in 22 patients, we measured an average postoperative fracture distraction of 1 cm. For many authors this may not be acceptable, but we found no difference in the outcome 6 months after surgery: indeed, from a radiological point of view, callus formation was slower in fractures with a bad reduction, and faster when a good reduction was achieved. However, from a clinical point of view, 73,3% of patients showed acceptable results, despite the radiological findings. In our study, clinical results were not influenced by factors such as the age of the patient, the length of the surgical procedure, the diameter of the intramedullary nai as well as the interfragmentary gap >6 mm. In fact, patients with greater distraction in

the fracture site showed less callus formation 3 months after surgery, but almost all patients showed acceptable callus formation at the 6 months follow-up. Only one patient out of 22 did not show radiological healing after 6 months.

A limit of this study was the low numerosity, with only 22 patients included due to strict inclusion criteria (over 65 years patients with a displaced humeral mid shaft fracture, treated with closed reduction and internal fixation with a locked intramedullary nail, with interfragmentary gap after surgery >6 mm) to avoid biases as much as possible.

In conclusion, osteosynthesis with anterograde nail in geriatric patients appears to be a quite safe approach despite a great interfragmentary gap. After 6 months of treatment, callus formation and the overall clinical outcome were proven to be above satisfaction.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Knowledge, attitudes, and practices of influenza and pneumococcal vaccines among agricultural workers: results of an Italian a cross-sectional study

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Summary. *Background:* Working age is increasing across Europe. Seasonal influenza (SID) and pneumococcal disease (PND) immunization programmes might be successfully implemented at the workplace. We conducted a cross-sectional survey among to assess SID and PND vaccine status, as well as knowledge, attitudes and practices (KAP) in a representative sample of agricultural workers (AWs) aged ≥55 years in North-Eastern Italy. *Methods:* A structured questionnaire was administered in person by trained personnel. Bivariate and multivariate logistic regression analyses were carried out to identify behavioral and work-related factors associated with SID and PND vaccine uptake. *Results:* Among 707 participants, 238 were aged 55 years or more (33.7% of total). Of them, 39.1% had an up-to-date immunization status towards influenza, and 17.6% towards pneumococcus. Factors associated with inadequate immunization were doubts about influenza vaccine safety (40.0%) and the confidence in natural immunity towards pneumococcus (30.8%). Attitude towards vaccinations was somehow favorable in 44.5% of participants for SID, and 37.8% for PND. Overall, 37.4% and 21.8% workers were aware of national recommendations on SID and PND immunization, respectively. This factor was characterized as a significant predictor for SID vaccination (multivariated Odds Ratio, OR 32.688 95%CI 12.015-88.930), as well as the perception of SID as a severe disease (OR 7.539 95%CI 3.312-17.164), and the perceived value of preventing new infections (OR 3.215 95%CI 1.205-8.578). A somehow favorable attitude towards vaccinations was the main predictor (OR 39.214 95%CI 10.179-151.1) for PND vaccination. *Conclusions:* Our study indicates that older workers lack appropriate knowledge of national recommendations and correct risk perception of SID and PND infections, but also vaccines' side effects. As the latter has been recognized as predictive factor for SID vaccination, our results stress the importance for tailored informative interventions in the workplaces aimed to increase risk perception and vaccine acceptance. (www.actabiomedica.it)

Key words: vaccines, vaccination refusal, vaccine hesitancy, seasonal influenza, pneumococcal diseases

Introduction

Influenza and pneumococcal disease are common vaccine-preventable causes of morbidity and mortality (1). Global annual attack rates for seasonal influenza range from 5 to 10% in adults, whose direct and indirect costs range from 6 to 14 billion € for the EU only (2). Even though severe influenza illness cases are relatively rare, influenza still remains a main trigger for lost workforce productivity (3-7). Also pneumococcal infection is a major cause of morbidity and mortality, both as community-acquired pneumonia

and invasive pneumococcal disease, i.e. pneumococcal invasion of normally sterile sites such as the bloodstream and meninges (8, 9). As older age groups are particularly vulnerable to severe outcomes, World Health Organization (WHO) and European Centre for Disease Prevention and Control (ECDC) strongly recommend seasonal influenza (SIV) and pneumococcal vaccination (PNV) in subjects older than 60 years (1, 2, 9-16). More specifically, the EU Council has recommended that member states achieve SIV coverage rates of 75% in subjects older than 65 years (2, 12), but results still remain suboptimal (13-15, 17), even in high risk groups (1, 9-12, 18). For instance, since 2014 Italian National Immunization Plan (NIP) recommends SIV for subjects 65 years of age or older, while some Italian regions, including Autonomous Province of Trento (APT) have issued similar recommendations regarding PNV (Resolution of Regional Council No. 2071/2014) (14, 19, 20). Both vaccinations are now regularly enforced by Italian NIP 2017-2019 (19, 20).

In recent years, the needs of the aging European workforce, characterized by an increasing share of workers aged 55 years or more, have raised the interest towards occupational policies including SIV and PNV (21-23). Unfortunately, although the uptake of influenza and pneumococcal vaccination in older workers and their determinants have been deeply investigated in healthcare workers (11, 24-26), very little is known about other occupational settings, and in particular for agricultural workers (AWs).

This particularly frustrating, as the 2010 European Union Farm Structure Survey showed that around 25 million people were still engaged in the primary sector, representing 4.6% of total employment, with a total workforce significantly older than most other occupational groups (27, 28). Moreover, even though assessment of *knowledge* (i.e. the awareness of official recommendations), *attitudes* (i.e. propensity towards vaccinations) and *practices* (i.e. actual uptake of vaccinations), or KAP, is acknowledged as necessary in order to a better planning of vaccine campaigns in target populations (29-32), few studies have specifically inquired AWs, in particular in Western Europe (33). Our primary objective was therefore to investigate the coverage rates for SIV and PNV in AWs from the highly developed agricultural settings of the APT (34). APT is located in the Italy's North East, covers a total area of 6,214 km^2 (2,399 sq. mi) and has a population of 537,416 habitants: at 2015 census, prevalence of subjects aged 55 years or more was slightly lower than national level (33.4% vs. 34.3%). According to labor force statistics, the primary sector in APT directly accounts for around 20,000 employees (dependent and self-employed) in 16,446 agricultural farms, including 11,958 agricultural enterprises, usually of small extent (89% are smaller than 5 hectares and 56% smaller than 1 hectare). These figures, however, do not include "hobby farmers" and part-time employees, whose number may largely exceed full-time employees (33-36). Our secondary objective was then to investigate KAP of AWs towards both vaccinations.

Methods

1. Study design and target population

The present investigation was a questionnaire-based cross-sectional study. The target population included AWs from the APT, and the sampling was performed through convenience, involving all consecutive participants to qualification courses held between January and June 2016, and focusing on the occupational use of pesticides (33, 35, 36). In the present study, following excluding criteria were applied: an inadequate ability to understand the Italian language, and age <55 years. A total of 252 consecutive AWs were eventually eligible to participate.

2. Instruments

The instrument used was a specifically designed structured questionnaire. The majority of survey items had been used in previous studies on KAP both in the general population (31, 32), and in occupational settings (29, 30, 33), but were adapted to our specific target population and design. Test-retest reliability of questionnaire items was preventively assessed by having 10 AWs complete the questionnaire at two different points in time. A correlation coefficient was calculated to compare the two sets of responses: items having a coefficient >0.80 were interpreted as consistent,

and were therefore included in the questionnaire used in this survey. All questions were self-reported, and not externally validated. The questionnaire included a total of 22 items divided into four areas of inquiry:

(*1) Information about the interviewee*. Retrieved data included: gender, age, educational level, birthplace (i.e. Italian-born people, IBP vs. Foreign-born people, FBP), main sources for information about health topics (i.e. TV/radio/newspapers, internet, friends, parents, school, healthcare professionals), and whether they had received or not any medical assessment in the previous 5 years, both in general and by an occupational physician.

(2) Knowledge about vaccines. Firstly, participants received a general knowledge test (31, 32), containing a total of 17 true-false statements such as "*vaccinations increase the occurrence of allergies*" (false), covering some typical misconceptions on vaccination and vaccination policies. General knowledge of participants was calculated through the sum of all appropriate answers. The sample was then categorized by mean value in participants having "*high knowledge*" (>median value) vs. "*inappropriate knowledge*" (≤median value). Eventually, participants were asked whether they were aware of official recommendations towards SIV and PNV for people aged ≥65 years, separately, allowing dichotomization in subjects aware vs. non-aware of official recommendations.

(3) Attitudes and Practices. Participants were initially asked to explain why they would get vaccinations (i.e. "*to avoid getting vaccine preventable diseases (VPDs)*", "*to avoid transmitting VPDs", "to avoid complications of VPDs*", "*to avoid VPDs in subjects who cannot be vaccinated*"). Specific propensity towards vaccinations was then assessed through a 5-point Likert scale (i.e. from "*strongly disagree*" to "*strongly agree*"), being ultimately dichotomized in somehow favorable attitude ("*agree*", and "*strongly agree*") vs. somehow contrary attitude (from "*neutral*" to "*strongly agree*"). Similarly, participants were then asked how severe they perceived natural infections in a 5-point scale ranging from "*almost zero*" to "*very high*"). Subsequent Risk Perceptions were eventually dichotomized as "*severe*" (i.e. "*high or rather high*"; "*very high*") vs. "*not severe*" ("*almost zero*"; "*low or rather low*"; "*moderate*") Participants were eventually asked about their immunization status for SIV and PNV: an appropriate SIV status was defined by having received a vaccination shot for seasonal influenza during the winter season 2015-2016, while an appropriate PNV status was defined by having received a vaccination shot for pneumococcus in the previous 10 years.

3. Ethical considerations

Before they give their consent, subjects to be inquired were informed that participation in the present survey was voluntary, and that the questionnaires would be gathered only from subjects expressing preliminary consent for study participation. Participants were guaranteed that they may withdraw from the survey in any time, by simply non delivering the questionnaire at the end of the course session, and that all collected information would be handled anonymously and confidentially. As the questionnaire was strictly anonymous, it is implausible that individual participants could be identified based on the presented material, and ultimately this study caused no plausible harm or stigma to participating individuals. Moreover, as the final examiners of professional courses were totally blind regarding the status of inquired subjects (i.e. whether they had participated or not in the survey), it is also highly unlikely that individual participants have been forced to give their consent. As the study design assured an adequate protection of study participants, and neither included clinical data about patients nor configured itself as a clinical trial, a preliminary evaluation by the Ethical Committee of the Provincial Agency for Health Services (APSS) was not required.

4. Data Analysis

Two independent researchers, one of whom read the responses from each questionnaire while the other reviewed the entered data, ensured the accuracy of data entry. The primary investigator examined unclear responses to determine the correct answer. Questionnaire lacking basic information about the interviewee were excluded from the study.

Continuous variables were expressed as mean ± standard deviation and were preliminarily tested for normal distribution (D'Agostino & Pearson omnibus

normality test): where the corresponding p value was <0.10, normality distribution was assumed as rejected and variables were compared through Mann-Whitney or Kruskal-Wallis test for multiple independent samples. On the other hand, variables passing the normality check (D'Agostino & Pearson p value ≥0.10) were compared using the Student's t test or ANOVA, where appropriate. Categorical variables were reported as per cent values and univariate confrontations between proportions were initially evaluated through Chi-squared test in order to examine correlates of self-assessed vaccination for SIV and PNV (updated vs. not updated) with individual data regarding age (dichotomized as <65 years vs. ≥65 years), sex, education (≤8 years vs. 9 years or more), birthplace, information sources, knowledge of official recommendations, knowledge status, risk perception and attitude towards vaccinations. In a binary logistic regression analysis model (SPSS 25, IBM Corp., Armonk, USA), we assessed the relative influence of individual factors on self-reported immunization status for SIV and PNV through calculation of multivariate Odds Ratios (ORs) with their respective 95% confidence intervals (95%CI). Regression models included as effectors all factors that at univariate analysis were significantly associated with an appropriate vaccination status for SIV and PNV. Significance level was < 0.05 for all calculations.

Results

1. Descriptive analysis of the interviewee

A total of 238 questionnaires were retrieved, with a response rate of 94.4% of the original sample. As shown in Table 1, 67.2% of the participants were aged 55 to 64 years: overall, 89.9% were males and 10.1% females, with 8.8% subjects from a migration background. All the participants had completed at least the primary education (5+3 y), and 52.5% of the respondents had achieved secondary education level (13 y) or higher. The most frequently referred information source was represented by a healthcare professional (70.2%), followed by conventional media (21.8%), friends and relatives (4.2%), whereas the least reported was represented by professional courses (2.1%). Overall, two thirds (67.6%) of participants had received a medical assessment in the previous 5 years, while 45.4% of AWs had been assisted by an Occupational Physician.

2. General knowledge

Internal consistency coefficient of the General Knowledge test (Table 2) amounted to Cronbach's alpha=0.878. Average rate of correct answer was 5.7±4.6 (i.e. 33.3%±26.8), with a median of 5 (29.4%) and actual range 0 to 16 (i.e. 0.0%-94.1%). Regarding the single statements, none of them was correctly recalled by 50% of more of participants. Not only majority of participants claimed the risks associated with vaccination practice are usually hidden from public knowledge, both in general (69.3%) and more specifically by healthcare workers (60.1%), but participants exhibited a significant share of misbelieves about severe vaccine side effects. Actually, 70.2% of them failed to identify severe side effects of immunizations as unusual, in particular as long-term side effects (73.9%), and were unable to recognize as incorrect claims about the risks associated with a high number of vaccines (75.6%), in particular when administered early in life (68.5%). Moreover, the majority of participants claimed for causative associations of vaccines with neurological diseases such as Multiple Sclerosis (76.5%) and autism (71.0%), in particular after measles vaccinations (74.8%), autoimmune disorders (73.9%) and diabetes mellitus (65.5%), but also with allergic disorders such as asthma and atopic dermatitis (66.8%). Uncertainties affected also positive effects of vaccines, as only 46.9% were aware that appropriate pharmacological treatments may be unavailable for all infectious diseases, and 42.9% that immunization elicited by vaccinations is not inferior to that following natural infection, whereas 46.2% correctly recognized that vaccinations were instrumental in smallpox eradication and 45.8% acknowledged that efficacy of vaccinations has been extensively proved.

3. Attitudes and Practices

Overall, 39.1% of respondents reported having been vaccinated against seasonal influenza during the

Table 1. Characteristics of 238 agricultural workers aged ≥55 years from the autonomous province of Trento (2016)

Variable	Total (No. = 238, 100%)
Age groups (No, %)	
55-59	96, 40.3%
60-64	64, 26.9%
65-69	46, 19.3%
70-74	28, 11.8%
≥75	4, 1.7%
Sex (No. %)	
Female	24, 10.1%
Male	214, 89.9%
Migration Background (defined by country of birth) (No., %)	
No	217, 91.2%
Yes	21, 8.8%
Education level (No., %)	
Primary/Secondary School	113, 47.5%
High School	96, 40.3%
University	29, 12.2%
Previous medical assessment (No., %)	161, 67.6%
Previous medical assessment by an Occupational Physician (No., %)	108, 45.4%
Main information source (No., %)	
Healthcare professional	167, 70.2%
Conventional media	52, 21.8%
Friends, relatives	10, 4.2%
Professional courses	5, 2.1%
New Media	4, 1.7%
Knowledge Status >median (No., %)	113, 47.5%
Knowledge of official recommendations (No., %)	
Pneumococcus	52, 21.8%
Seasonal Influenza	89, 37.4%
Natural infection perceived as a severe one (No., %)	
Pneumococcus	108, 45.4%
Seasonal Influenza	87, 36.6%
Somehow favourable towards vaccine (No., %)	
In general	178, 74.8%
Pneumococcus	42, 17.6%
Seasonal Influenza	93, 39.1%
Appropriate vaccination status (No., %)	
Pneumococcus	42, 17.6%
Seasonal Influenza	93, 39.1%
Vaccines avoid (No., %)	
VPD infections	184, 77.3%
VPD transmission	59, 24.8%
VPD complications	50, 21.0%
Communicable VPDs in subjects who cannot be vaccinated	12, 5.0%
Perceived barriers towards vaccinations (No., %)	
Fear of acute side effects	50, 21.0%
Fear of chronic side effects	29, 12.2%
Distrust in Public Health Providers	44, 18.5%
Distrust in National Health Service	36, 15.1%
Difficulties in interaction with Public Health Providers	114, 47.9%
Difficulties in accessing Public Health providers	68, 28.6%
Ethical / Religious barriers	35, 14.7%

Table 2. Knowledge test of 238 agricultural workers aged 55 years or more, from the Autonomous Province of Trento (2016) (31-32)

Statements	Correct answer	No. (%)
1. Additives used in the vaccines are not dangerous for humans	True	82 (34.5%)
2. Neurologic diseases such as Multiple Sclerosis may be induced by HBV vaccine	False	56 (23.5%)
3. Diabetes mellitus may be triggered by vaccination shoots	False	82 (34.5%)
4. Vaccinations increase the occurrence of auto-immune diseases (e.g. rheumatoid arthritis, some thyroid diseases etc.)	False	62 (26.1%)
5. Autism is more frequent in subjects vaccinated against measles	False	69 (29.0%)
6. Severe diseases of the CNS are a possible side effect of measles vaccine	False	60 (25.2%)
7. Vaccinations increase the risk for allergic disorders such as asthma and atopic dermatitis	False	79 (33.2%)
8. Appropriate pharmacological treatments are available for all infectious diseases	False	118 (49.6%)
9. Without massive vaccination programs, smallpox would still exist	True	110 (46.2%)
10. Efficacy of vaccination has been extensively proved	True	109 (45.8%)
11. Children would be more resistant to infections if they were not always treated against all diseases	False	100 (42.9%)
12. Many vaccinations are administered too early. As results, the immune system has no possibility to fully develop by itself	False	75 (31.5%)
13. The immune system may be overwhelmed by a high number of vaccines	False	58 (24.4%)
14. Severe vaccine side effects are usually hidden from public knowledge	False	73 (30.7%)
15. Severe vaccine side effects are unusual	True	71 (29.8%)
16. Severe side effects may develop also several years after vaccination shots	False	62 (26.1%)
17. Healthcare workers usually hidden the risks associated with vaccination practice	False	95 (39.9%)

winter season 2015-2016, while only 42 AW (17.6%) recalled previous PNV. As shown in Figure 1, no significant trend through age groups was recognized. Higher shares for updated SIV status were reported in age group 65-69 years (45.7%), with the lower in older age groups (39.3% and 25.0% in age groups 70-74 and ≥75 years, respectively). Conversely, 65-69 years was associated with lowest rates for PNV (4.3%), while highest rates were identified in age groups 60-64 years (32.8%) and 70-74 years (28.6%).

Focusing on attitudes of participants while the majority of them exhibited a somehow favorable attitude towards vaccines: among the reasons to be vaccinated, the majority of participants recalled prevention of VPD infections (77.3%), followed by avoiding VPD transmission (24.8%), their complications (21.0%), whereas only 5.0% advocated prevention of VPD among people that cannot be vaccinated. However, only 106 participants exhibited a favorable attitude for SIV (44.5%), and 90 AWs were somehow favorable towards PNV (37.8%). Among the barriers towards vaccinations, nearly half of participants complained difficulties in interaction with Public Health Providers (e.g. having explanations about vaccine efficacy and efficiency; how to cope with side effects, etc.), followed by difficulties in accessing Public Health providers (28.6%), fear of acute side effects (21.0%), distrust in Public Health Providers (18.5%) as well as in the National Health service (15.1%), perceived ethical/religious barriers (14.7%), and eventually fear of chronic effects (12.2%).

5. Statistical analysis

In univariate analysis (Table 3), updated PNV and SIV status was significantly associated with a previous

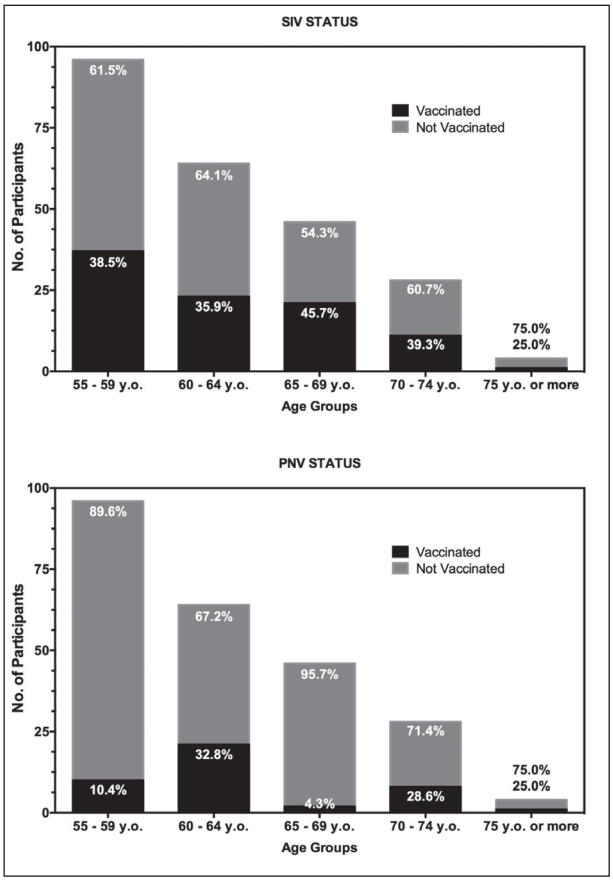

Figure 1. Vaccination status for pneumococcus (PNV) and seasonal influenza (SIV) by age groups in 238 agricultural workers from the Autonomous Province of Trento (2016)

medical assessment by knowledge of official recommendations, perception of natural infections as severe diseases, and a favorable attitude towards vaccination, both in general and specifically toward the specific immunization. Moreover, PNV was specifically association with a higher education level (p=0.018) and having received a medical assessment in the previous 5 years (p=0.017), while willingness to prevent VPD transmission was statistically associated with an updated SIV status (p=0.022). Among perceived barriers towards vaccinations, distrust in National Health Service (p=0.015) and ethical reasons (p=0.025) were significantly associated with a not appropriated PNV status, while inappropriate SIV status was associated with logistical issue (i.e. accessing Public Health providers).

In regression analysis, significant effectors of updated SIV status were identified in knowledge of official recommendations for people ≥65 years (OR 2.662, 95%CI 1.005-7.047), perception of seasonal influenza as a severe disease (OR 7.539, 95%CI 3.312-17.164), and a positive attitude towards vaccination, both in general (OR 6.995, 95%CI 2.895-16.899), as well as specifically for SIV (OR 32.688, 95%CI 12.015-88.930). Focusing on PNV status, a positive attitude towards vaccinations was identified as positive drivers for an updated status (OR 7.996, 95%CI 1.815-35.234 and OR 39.214, 95%CI 10.179-151.1 for vaccinations in general and for PNV, respectively). On the contrary, distrust in National Health Service was identified as negative effectors for an updated PNV status even at multivariate analysis (OR 0.329, 95%CI 0.125-0.867).

Discussion

Our study aimed to estimate SIV and PNV rates and determinants of vaccination status in a sample of AWs aged ≥55 years from North-Western Italy. Despite increasing efforts, vaccination rates for seasonal flu and pneumococcal disease are diffusely disappointing, particularly in Italy (9, 14, 15, 19, 37, 38). For instance, official data suggest that, during winter season 2015-2016, overall SIV rate was around 13.9%. Even though older age groups exhibited higher share for an updated SIV status (i.e. 49.9%), available figures remain largely below the European targets (14). Also APT has been affected by such lack of confidence towards SIV and PNV. On the one hand, during 2015-2016, only 24.3% of residents older than 45 years had received SIV, peaking to 50.2% among subjects older than 65 years. On the other hand, not only the vaccination rates for PNV remained well below 50% of the target population (i.e. 48%), but a negative trend since the beginning of the decade was eventually reported (39).

Our data are somewhat conflicting with national and regional estimates. Vaccination rate for SIV was somewhat higher than that reported in general population (i.e. 39.1%), and a specular ratio between age groups was noticeable, as the share of AWs who had reportedly received SIV was 41% among subjects 55 to 64 year-old, compared to 28.0% among older participants. On the contrary, the share of participants

Table 3. Association between updated vaccination status for Pneumococcus (PNV) and Seasonal Influenza (SIV) and individual characteristics of 238 agricultural workers from the Autonomous Province of Trento (2016). Odds Ratios (OR) and their respective 95% Confidence Intervals (95%CI) were calculated through binary logistics regression analysis including factors significantly associated with updated self-assessed PNV / SIV status at univariate analysis (Note: VPD, vaccine preventable disease; OPh, Occupational Physician)

	Updated self-assessed PNV N = 42, 17.6%			Updated self-assessed SIV N = 93, 39.1%		
	No., %	P value	OR (95%CI)	No., %	P value	OR (95%CI)
Age >65 years	11, 26.2%	1.000	-	26, 28.0%	0.883	-
Male sex	35, 83.3%	0.118	-	86, 92.5%	0.294	-
Education level ≤ 8 years	13, 31.0%	0.018	0.278 (0.105; 0.737)	47, 50.5%	0.449	-
Migration background	2, 4.8%	0.306	-	11, 11.8%	0.191	-
Previous medical assessment	35, 83.3%	0.017	1.918 (0.664; 5.542)	62, 66.7%	0.796	-
Previous assessment by an OPh	21, 50.0%	0.623	-	46, 49.5%	0.379	-
Known recommendation for ≥ 65 years	21, 50.0%	<0.001	1.132 (0.379; 3.380)	45, 48.4%	0.005	2.662 (1.005; 7.047)
High Knowledge status	19, 45.2%	0.881	-	47, 50.5%	0.553	-
Perception as a severe disease	23, 54.8%	0.007	0.975 (0.375; 2.543)	50, 53.8%	<0.001	7.539 (3.312; 17.164)
Attitude somehow favourable towards vaccines (in general)	40, 95.2%	0.002	7.996 (1.815; 35.234)	84, 90.3%	<0.001	6.995 (2.895, 16.899)
Attitude somehow favourable towards specific vaccine	38, 90.5%	<0.001	39.214 (10.179; 151.1)	73, 78.5%	< 0.001	32.688 (12.015; 88.930)
Information sources						
Healthcare professionals	38, 90.5%	1.000	-	69, 74.2%	1.000	-
Conventional Media	0, -	-	-	20, 21.5%	0.714	-
New Media	0, -	-	-	1, 1.1%	0.521	-
Friends, relatives	0, -	-	-	2, 2.2%	0.201	-
Professional courses	1, 2.4%	0.885	-	1, 1.1%	0.359	-
Vaccines avoid						
VPD infections	31, 73.8%	0.550	-	78, 83.9%	0.053	-
VPD transmission	13, 31.0%	0.411	-	31, 33.3%	0.022	0.937 (0.412; 2.130)
VPD complications	12, 28.6%	0.185	-	21, 22.6%	0.633	-
VPDs in subjects who cannot be vaccinated	0, -	0.209	-	7, 7.5%	0.272	-
Perceived barriers						
Fear of acute side effects	7, 16.7%	0.581	-	22, 23.7%	0.522	-
Fear of chronic side effects	5, 11.9%	1.000	-	15, 16.1%	0.198	-
Distrust in Public Health Providers	7, 16.7%	0.908	-	19, 20.4%	0.655	-
Distrust in National Health Service	12, 28.6%	0.015	0.329 (0.125; 0.867)	18, 19.4%	0.203	-
Difficulties in interaction with Public Health providers	26, 61.9%	0.067	-	49, 52.7%	0.293	-
Difficulties in accessing Public Health providers	10, 23.8%	0.572	-	34, 36.6%	0.042	1.786 (0.866; 3.686)
Ethical / Religious barriers	1, 2.4%	0.025	0.485 (0.218; 1.079)	12, 12.9%	0.659	-

reporting a previous PNV shot was even lower than regional figures (i.e. 17.6% for total sample, 26.2% among age group ≥65 year-old) (14, 38-40).

Such data may found some explanations in vaccine and disease specific characteristics, as well as in personal factors, that have been collectively identified

as important effectors in the individual decision making process to accept vaccination of person aged 50 years and older (40-42).

First at all, participants we deliberately sampled were still leading an active life, working either as hobby farmers or professional farmers, and previously reports suggest that subjects who rate their own health status as low or somehow unsatisfying are more likely to receive SIV and PNV compared to a good subjective health status (40-44). Moreover, our study presumptively underrepresented subjects affected by age-related comorbidities (e.g. cardiovascular disorders, respiratory disorders, etc.) that usually mean strong recommendations towards SIV and PNV. In addition, the very same study design also deliberately excluded institutionalized populations, usually targeted by official recommendations, and consequently characterized by very high vaccination rates (41-43). Not coincidentally, a usual barrier towards vaccinations represented by accessibility issues was reported by a relatively reduced share of participants (28.6% of total sample), being ultimately not associated with an inappropriate vaccination status (25, 40-42, 44-46).

Second, the majority of participants exhibited an inappropriate knowledge status, with an insufficient awareness of official recommendations for older age groups. This is of particular interest, as elderly adults who score more highly on knowledge questions are more likely to be vaccinated. Particularly older adults that perceive themselves as healthy, usually prefer prevention against diseases they understand as associated with high mortality rates and high vaccine effectiveness: disregarding actual risks associated with seasonal influenza and pneumococcal infection may therefore affect vaccination rates even more extensively than in younger subjects (40, 41, 43, 44, 47, 48).

Even though previous reports have suggested that perceived severity is weakly correlated with vaccination acceptance, perceiving influenza as a severe disease was identified as a significant effector of the vaccination status (OR 7.539, 95%CI 3.312-17.164), while no significant correlation was found with PNV after multivariate analysis (40-42, 48).

Moreover, available evidence suggests that being aware of vaccine recommendations is strongly associated with vaccine uptake, and also in our study a better awareness was identified as a significant effector of SIV uptake (OR 2.662, 95%CI 1.005-7.047) (42, 44, 48, 49). On this regard, it is of significant interest that only a fourth of participants was reportedly aware of specific advices for PNV, with a nearly double share of AWs that acknowledged similar SIV recommendations (21.8% vs. 37.4%). A possible explanation may found in the heterogeneous nature of specific recommendations before the enforcement of NIP 2017-2019. When the survey was performed, SIV in subjects older than 65 years was implemented by national guidelines, whereas PNV had been introduced only recently, in 2014, and only through a Provincial decree, ultimately limiting the possible awareness of both patients and providers (9, 14, 37, 40).

Third, in our sample overall vaccination propensity was the main effector for an updated vaccination status, but overall figures were somehow unsatisfying. Although around 75% of participants self-assessed as favorable or even highly favorable towards vaccinations, a positive attitude was identified only in 17.6% and 39.1% of them for PNV and SIV, respectively. As recently suggested, behavioral intention may be modeled by specific beliefs and individual evaluation of possible outcomes (44, 48, 49): in this regard, even though 77.3% of participants associated vaccination practice with prevention of VPD infection, other significant drivers such as avoiding VPD transmission, their complications, as well as prevention of VDP in people who cannot be vaccinated, were reported by less than one fourth of sampled AWs. Similarly, our sample was affected by high or relatively high share of perceived barriers and misbeliefs about pros and cons of vaccinations, as well as regarding vaccination services. PNV status in particular was significantly affected by distrust in National Health Service.

Interestingly enough, the potential role of healthcare providers was somewhat conflicting with available evidences. Several reports have suggested that clinician reminders and specific recommendation by a healthcare providers are among the stronger effector for vaccine uptake, particularly for PNV. In other words, information provided by healthcare providers, as well as their throughout support in the promotion of vaccination among older age groups has the potential to significantly improve vaccination propensity (50, 51).

However, even though 70.2% of participants identified healthcare providers as their preferred information source, and a similar share reportedly interacted with healthcare providers on a regular basis, such factors were unrelated with vaccination status. We could speculate that the positive effector represented by interaction with healthcare providers may have been hindered by a diffuse distrust in National Health Service and Health professionals, as well as by the perceived difficulties reported in the interaction with healthcare providers (47.9%). Not coincidentally, referred distrust in National Health Service was a significant effector for an inappropriate PNV status.

However, our study is affected by several limitations. Firstly, vaccination status was self-reported, and subsequent figures may be affected by a significant recall bias and social desirability bias as well. In other words, our results might be affected by an implicit misreporting, because of individuals' recall errors on the one hand, and subjects' answering to questions in a manner that will be viewed favorably rather than factually, on the other hand (52, 53). However, evidence from previous occupational studies hints that questionnaire-based self-reporting of vaccinations usually guarantees a relatively accurate measure of vaccination status (54).

Second, it should be stressed that our sample was of relatively small size, being gathered through convenience sampling and a regional basis, and Italy is highly heterogeneous in terms of socio-economical development, with striking differences in terms of vaccination coverages (14, 19, 55). Moreover, also the study population, i.e. AWs performing pesticide application, included only subjects having a relatively high qualification, both in term of personal education and in empirical experience with farming practices: as a consequence, generalization of our results may be cautiously applied only to similarly highly developed agricultural settings (33, 35, 55, 56).

In conclusion, our study suggests that older workers (i.e. workers aged 55 years or more), and particularly AWs, may exhibit KAP towards SIV and PNV somehow conflicting with available evidences drawn from the general population. The main reason for such heterogeneity may be identified in the relatively healthy, active status of participants deliberately included in this study. Our results were also affected by the ongoing loss of confidence in healthcare professionals and Italian National Health System, stressing the importance of interventions aimed to restore perceived truthfulness on healthcare providers and public health services. On the other hand, the large share of participants who reported to regularly interact with healthcare providers suggests that improving vaccination literacy among older workers, focusing on the awareness of official recommendations as well as the actual risk associated with natural infections, might have a beneficial effect on vaccination coverages.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Five year long term result of total joint arthroplasties in the treatment of trapeziometacarpal osteoarthritis

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Summary. *Introduction:* Trapeziometacarpal (TMC) osteoarthritis is a common disease. Patients with advanced disease who have failed conservative treatment have different surgical options, including total joint prosthesis. The aim of this study was to investigate the long-term outcome and complications of trapeziometacarpal (TMC) total arthroplasty. *Materials and Methods:* One hundred and forty-seven patients with TMC osteoarthritis were surgically treated with TMC arthroplasty, and one hundred and thirty-seven patients were seen for follow-up (102 women and 35 men). At follow -up patients were asked to complete a visual linear-analogue scale (VAS) for satisfaction with the result of the operation and persisting pain from the thumb, the Spanish validated Disabilities of the Arm, Shoulder and Hand (DASH) questionnaire was used to evaluate function of the affected hand.(9) The radiological examination consisted of posterior-anterior and oblique radiographs. In preoperative radiographs Eaton-Little was used, and in postoperative radiographs were assessed with regard to implant loosening and alignment. *Results:* The subjective outcome was satisfactory in 126 cases (92 %), 14 (12 %) patients would undergo the same procedure in the other hand. The DASH questionnaireswere 19.55 (range 5.6-33,5) on average, and EVA was 1 on average. The mean key pinch strength was 5.8 Kg at 5 years follow-up. The most frequent postoperative complication was De Quervain tenosynovitis (21%), other complications were: Cup loosening (3.6%), traumatic dislocation (3,6%). The prosthesis was removed in nine cases (7%). There were four intraoperative complications. The survival rate for ARPE prosthesis was 92,7% at 60,5 months. TMC total arthroplasty offers a reliable treatment alternative in patients with thumb carpometacarpal joint osteoarthrosis which conservatives' treatment had failed. *Conclusions:* The TMC joint prosthesis is an option for patients with TCM osteoarthritis, provides satisfactory outcomes and has a low failure rate.(www.actabiomedica.it)

Key words: thumb, arthritis, metacarpal, finger, joint, outcome, DASH, prosthesis

Introduction

The first carpometacarpal joint of the thumb given the myriad of activities that an average human undertakes. It can move in three main planes: abduction-adduction, flexion-extension and opposition. The main ligamentous stabilizers of this articulation are the anterior oblique ligament and the dorsoradial ligament.

Trapeziometacarpal(TMC) osteoarthritis is a common entity (1). The eventual degenerative change of TMC joint including joint space narrowing, osteophyte formation, ligament attenuation and dorsal radial subluxation of the joint (2).

TMC osteoarthritis is a common disease in postmenopausal women, leading to severe disability an pain (1, 3), Prevalence reported as high as 15% in older than 30 years and precise causes remain unclear (4). The typical presentation is insidious radial thumb pain that it is worsened with use, has decreased her ability to perform daily activities, decreases strength and dexterity. There has been an increasing stiffness and cramping with increasing stage (2). Physical examination often show a dorsoradial prominence of the first metacarpal base, pain is usually focus in trapeziometacarpal joint and crepitus may often be felt, and the grind test of the trapeziometacarpal joint will often produce pain (2).

Radiographs of the TMC joint in the standard anteroposterior, 30° anteroposterior, lateral and oblique view help confirm the diagnosis. But not only radiographic evidence is enough in order to make the diagnosis, since approximately 25% of women and 8% of men will develop radiographic evidence of TMC osteoarthritis, and only 28% of this woman will admit to pain (2).

Several different methods exist for the radiographic staging of TMC arthritis. Eaton-Littler classification system is currently most widely used for TMC osteoarthritis staging (4) (Table 1).

Conservative treatment including NSAIDs and splinting is mostly successful, and surgical treatment is only indicated in resistant cases (1, 3).

Surgical options vary with the stage and nature of the disease. Multiple surgical procedures have been introduced since simple excision reported in 1949 by Geravis (5), include fusion, thumb metacarpal extension osteotomy, simple excision of the trapezium, trapeziectomy combined with interposition arthroplasty and total joint replacement.

The two most frequently used techniques currently are implant arthroplasty and trapeziectomy with ligament reconstruction and tendon interposition (LRTI). Although a prospective study by Martinez-Martinez et al comparing LRTI with implant arthroplasty, demonstrated no significant differences in Visual Linear Analogue Scale (VAS), Disabilities of the Arm, Shoulder and Hand (DASH) and pinch grip strength at 12-moth follow up.(6)Many authors continued to recommend trapeziectomy with ligament reconstruction and tendon interposition (LRTI) as the gold standard for surgical treatment, since high complication rates of implant arthroplasty and the need for more long-term studies (7).

Table 1. Classification of trapeziometacarpal joint arthritis

Stage	Characteristics
I	Slight widening of the joins space, less than 1/3 subluxation
II	Narrowing of the trapeziomecarpal joint and osteophytes or loose bodies less than 2 mm in diameter
III	Marked decrease in the trapeziometacarpal joint space, subchondral cyst, osteophytes or loose bodies biger than 2 mm
IV	Degeneration involves the scaphotrapezial joint.

A number of different joint replacements have been developed including spacers, hemiarthroplasties and total joint replacements. The ARPE TMC joint total arthroplasty was introduced in 1991, it has become one of the established treatments for this disease, and ARPE TMC joint total arthroplasty have been reported 10-year survival rated of 94% with high patient satisfaction (8).

The purpose of this retrospective study was to determine the complication rate, the survival rate and patient satisfaction for a consecutive series of patients undergoing primary trapeciometacarpal total arthroplasty.

Patients and methods

In 147consecutive patients with osteoarthritis of the trapeziometacarpal joint 151 (14 bilateral case) thumbs were surgically treated at Hospital Virgen de la Salud (Toledo, Spain) between 2013 and 2014. The indication for surgery was failure of conservative treatment in patients with TMC. 137 patients were seen for follow-up, 2 patients had died, and 18 patients were lost to follow -up.

Patients included in the study had undergone TMC joint arthroplasty for primary osteoarthritis, and conservative treatment fails, including steroid injections and physical therapy for a minimum of 6 to 12 weeks.

The ARPE TMC joint arthroplastyconsists of an unconstrained uncemented arthroplasty with a cup inserted into the trapezium and a stemmed component inserted into the thumb metacarpal.

At follow-up patients were asked to complete a visual linearanalogue scale (VAS) for satisfaction with the result of the operation and persisting pain from the thumb, the Spanish validated Disabilities of the Arm, Shoulder and Hand (DASH) questionnaire was used to evaluate function of the affected hand (9). The radiological examination consisted of posterior-anterior and oblique radiographs. In preoperative radiographs Eaton-Little was used, and in postoperative radiographs were assessed with regard to implant loosening and alignment.

Surgical technique

A lateral approach was used in all cases, with the incision centred over the TMC joint, the extensor pollicis brevis was reflect dorsally and the abductor pollicis longus was reflected ventrally. The capsule of the TMC joint was opened longitudinally, and saw was used to cut off 6-8 mm of the proximal surface of the first metacarpal perpendicular to the metacarpal axis.

And awl was used to open the intramedullary canal of the first metacarpal bone and enlarged with rasps until satisfactory cortical contact was obtained. The trial stem was implanted, and the back of the stem was aligned with the nail of the thumb.

The fist metacarpal was then subluxate ventrally to provide access to the trapezium, a thin bone cut was made to remove a little bone as possible. The osteophytes were removed.

The centre of the trapezium in distal surface was identified and a hole was drilled with surgical awl, this hole was enlarged with curettes and trapezium reamer.

A trial head was inserted, and a trial reduction undertaken, the trail was assessed for stability and impingement. The definitive head was inserted and the joint reduced. The capsule and the skin were closed (Figure 1).

Figure 1. Exemple of trapeziometacarpal prosthesis

A short plaster of Paris arm cast in functional position for 3 weeks was used in all patients. After 3 weeks the cast was removed, and an exercise programme was undertaken.

The Kaplan-Meier method was used to estimate survival probability over time.

All surgeries were performed by the same experienced and fully qualified orthopaedic had surgeon, and at follow-up the patients were evaluated by Italian independent researchers.

Result

In all, 137 patients (151 thumbs) were reviewed at a mean of 60,5 months (range 55-66), after the procedure. Most of the patients were women: 102 women and 35 men. The mean age was 61,6 years (range 46-78 years) at the time of the surgery. The dominant hand was affected in 79% of patients and 38% had already undergone trapeziectomy with LRTI (Zancolli technique) for basal joint arthritis in their contralateral hand.83 right thumbs and 54 left thumbs were treated; 91 of the treated thumbs were dominant (Table 2).

129 number nine cups were implanted in the trapezium and 8 number ten,97 number nine and 40

Table 2. Description of population

137 Patients	151 Thumbs
102 Female	35 Male
83 Righ hand	54 Left Hand

number ten stems were implanted in the first metacarpal. An angulated neck was used in 122 case and 15 straight neck was used.

The Eaton-Littre grade(4) was: Grade II in 2 cases, grade III in 101 cases and grade IV in 34 case.

The subjective outcome was satisfactory in 126 cases (92%), 14 (10%) patients would undergo the same procedure in the other hand. The DASH questionaries was 19.55 (range 5.6-33,5) on average, and EVA was 1 on average. The mean key pinch strength was 5.8 Kg at 5 years follow-up.

There were a high rate 29 cases (21%) of De Quervain`s tenosynovitis, these complications occur around the 3rd month and it was recovery with conservative treatment.

77% demonstrated no evidence of loosening or subsidence. 13% demonstrated changes. Cup loosening in five case (3,6%). The other radiologically changes were minor abnormalities without clinical impact like slight mal positioning of components, partial areas of radiolucency around cup or and heterotopic bone around de joint.

The prosthesis was removed in nine cases (7%) six at five years and three at four years. A trapezectomy and ligament reconstruction and LTRI (Welby technics) was performed in all case of prosthesis loosening with clinical impact.

There were four intraoperative trapezial fracture (3%) that occurred on impaction of the cup, immobilization for 6 weeks was enough for three patients and in one case trapezctomy and LRTI was necessary since impaction of the cup was not possible.

Five (3,6%) traumatic dislocation occurred along five years, in three cases close reduction and immobilization was undertaken for 3 weeks and the normal function was recovered, and in two case the cup was loosening, and new surgery was performed (Welby Technic).

The survival rate for ARPE prosthesis was 92,7% at 60,5 months.

Discussion

Osteoarthritis of the TCM joint is a frequent disease in the elderly and in postmenopausal women, can cause severe pain, weakness, and marked disability (3, 10).

In the case that nonsurgical treatment fails, many surgical options have been described for the treatment of severe TMC arthritis.(3)(11) However, there is no evidence that one technique is better than another (7, 10.

Simple excision was first reported in 1949 by Gervis, with good results in eighteen operations in fifteen patients. Simple trapeziectomy is an easy and quick procedure (5).

In more recent reports, LRTI arthroplasties are popular, and it is considered to be the gold standard for surgical treatment of TMC osteoarthritis(1)In the randomised studiers which compare trapeziectomy alone or combined with LRTI, no statistical difference was found (12, 13). Trapeziectomy with LRTI resulted in similar grip strength as simple trapeziectomy, the surgical technique led to a significant loss in pinch strength and significantly higher disability (DASH) score (14).

Thumb metacarpal extension osteotomy was described by Wilson in 1973, Artroshi et al, compared trapeziectomy with LRTI with metacarpal extension osteotomy, and concluded that metacarpal osteotomy should be limited to patients with early stage (15).

TMC arthrodesis is an effective alternative to LRTI procedure for patients with high physical demand. Fusion of the TMC articulation proved to be a reliable surgery with good long term results, different methods of fixation have been described in the literature The main problem of the arthrodesis technique is the non-union and hardware failure, although non-union not always are symptomatic(1). Kazmer et al, compared 14 patients treated with modified arthrodesis versus 22 LRTI patients, and found 25% greater pinch strength in fusion group, as well as similar range of motion, the non-union rate was of 7 % (16). Non-union rates in literature are on average 8% to 21% (7).

Another newer alternative technique is suspensionplasty with Mini TightRope and hemitrapeziectomy, it is a new arthroscopy technique. Some retrospective study found good outcomes, but there is little long-term data on the use this surgical procedure (17).

Total TMC joint replacement surgery was developed in 1973 by De la Caffinière, since the first de-

scription many designs of cemented and uncemented prostheses have emerged (18).

In the 2008s Ulrich-Vinther et al compared the Elektra joint prosthesis with trapeziectomy and LRTI, and reported that patients with joint prosthesis achieved faster recovery, more comfort, better strength and better range of motion without and increased complication rate (19).

Martin-Ferrero et al, studied the 10-year long-term results of the ARPE prosthesis in 64 patients, and report 10 year survival rate of 93.9%, with median VAS score at 10 year was 1.1 and the mean key pinch strength 5.6 kg (8). This resultare comparable with our study.

Bricout et al, reported survival rate of the Maia prosthesis of 91% at 62 moths, and subjective satisfactory outcome in 87% of patients. They also could detect risk factors for surgical revision, as presence of preoperative thumb deformity, poor trapezium bone quality discovered during the surgery and incorrect positioning of trapezial cup (20).

De Quervain tenosynovitis after TMC arthroplasty in the first years is a reported complication. Goubau et al reported 17% rate of De Quervain disease (21) and Bricout et al 16% (20). This rateis similar in this report.

Major complications are not too frequent, and revision surgery rate usually is less than 10% in the recent literature. Trapeziectomy with LRTI is considered an option in revision case, with worsened outcome and unpredictable compared with primary LRTI surgery. (22, 23)

The TMC joint prosthesis is an option for patients with TCM osteoarthritis, provides satisfactory outcomes and has a low failure rate. The main complications were De Quervain tendinopathy, trapezial cup loosening and prosthetic dislocation, but not all complication required revision surgery since the clinical status could be successfully.

If revision surgery is needed, trapeziectomy and LRTI is still an option, while TMC arthroplasty in revision surgery of trapeziectomy and LRTI is not possible.

Human and animal right: For this type of study is not required any statement relating to studies on humans and animals. All patients gave the informed consent prior being included into the study. All procedures involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Occupational Eye Injury in the agricultural settings: a retrospective study from North-Eastern Italy

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Summary. *Background:* Occupational Eye Injury (OEI) represents a common world-wide event accounting for between 3.3% and 6.1% of all occupational compensation claims. In this retrospective study we evaluated all the recorded OEI which occurred in the Autonomous Province of Trento (APT) during the period 2000-2013. *Methods:* Data on OEI for all of APT were retrieved an institutional archive and the analysis included demographics of the injured, as well as characteristics and settings of the OEI. In order to assess the risk of OEI in Agricultural Workers (AWs) vs. all other Occupational groups, a multivariate analysis was eventually performed through a logistic regression analysis. *Results:* A total of 141,139 work-related injuries were recorded, including 5,065 (3.6%) OEI. 91.9% of all cases occurred in males, of Italian origin (77.2%), with a mean age of 38.4±11.7 years. The industrial sector reported the higher share of OEI (70.7%), whereas higher incidence rates were reported among AWs (6.04 vs. 3.85/1,000 workers/year). Agricultural OEI occurred in older workers (45.6±13.3 vs. 37.1±11.0 years), being more likely associated with "contusions" (OR 2.042, 95% 1.602-2.602) and "lacerations" (OR 2.386, 95%CI 1.877-3.033), and less frequently with exposures to chemicals, gases and vapours (OR 0.478, 95%CI 0.279-0.817). *Conclusions:* Despite a relatively low frequency of OEI, AWs were affected with a seemly higher incidence than that reported in other occupational groups. OEI in AWs exhibited a specific pattern, both in terms of lesion, and settings of the events, recommending tailored interventions in order to improve promotion strategies. (www.actabiomedica.it)

Key words: eye injuries, occupational injuries, farmers, wounds and injuries

Introduction

Even though the eye represents only 0.27% of the total body area and 4% of the facial area, Occupational Eye Injury (OEI) is a common world-wide event (1-12), with a share of subsequent residual visual loss (RVL) disproportionately high compared to non-work related eye injuries. As up to 5% of all blindness may be due to work related injuries (4, 10, 13), OEI remains a significant cause of visual morbidity, with a significant socioeconomic impact because of the subsequent impairment of life quality and the reduction of work ability, ultimately representing a global public health concern (1, 4, 8, 14, 15).

Global estimates are largely heterogeneous, but data from highly industrialized countries suggest that OEI accounts for between 3.3% and 6.1% of all workers compensation claims, and for 30% to 70% of all the accesses to the Ophthalmological Emergency Departments (1, 2, 5, 8, 14, 16), but these data may be under-

estimated, as many trivial OEIs are usually unreported (1, 17). Interestingly enough, available data suggest that OIE may involve not only the worker actually exposed to the risk factors, but also bystanders, stressing the importance of both personal and collective preventive measures (18).

As a significant share of cases apparently does not wear PPE at the time of injury (4, 10, 13, 17), potentially peaking to 80% in certain economic sectors such as construction industries (18), OEIs are reputed largely preventable through a strict compliance on the use of proper Personal Protective Equipment (PPE; i.e. safety goggles, face shields, helmets) (10, 13, 14, 18-20). Hence, prevention of OEI may represent a significant contribution to the Action Plan devised by the 66th World Health Assembly for the reduction of avoidable visual impairment (21).

Although available data are very heterogeneous, as the epidemiology of OEI is not consistently documented around the world (9, 15, 17), no occupational sector appears immune to the risk of OEI, and more than one half of total injuries would occur in the manufacturing and construction industries (9, 18): mechanics, particularly welders, are considered to be at particularly high risk due to the exposure to harsh working conditions and a number of risk factors (i.e. dusts, UV radiations, metal parts, and chemicals), and their life-long prevalence of OEIs may be up to 50% (13, 17). Also agricultural settings are usually considered at high risk (17), but available data are fragmentary (8), with a considerable lack of evidence from South-Western European regions where farming is highly developed and profitable, and occupational health and safety practices are strictly regulated. Therefore, specific epidemiological studies may be useful in order to improve our understanding of this occupational risk (15, 22).

For these reasons, we have retrospectively evaluated all the recorded OEIs which occurred in a North-Eastern Italian Region characterized by highly developed agricultural sector, namely the Autonomous Province of Trento (APT). As the available time spans 14 years (2000-2013), our analyses included further assessment on the trends of OEIs, as well on their frequency, type, and other relevant characteristics, in particular cases of OEI with significant RVS.

Methods

1. Settings. APT is located in the Italy's North East, covers a total area of 6,214 km^2 (2,399 sq. mi) and has a population of 537,416 habitants (2015 census). The territory is overwhelming mountainous (70% over 1,000 m, and 20% is over 2,000 m), and APT may be ultimately defined as a cluster of side valleys "held together" by the Adige river and Trento-Rovereto road, which is the main communication route within the area and with the outside world. In the last decade, economic performances of APT have outperformed that of Italy, with a limited decrease of its GDP per capita (-3% in 2009 compared to Italy's -5%), that significantly exceed national (+22% in 2010) average. Despite a recent rise, unemployment rate in APT remains significantly lower than national average (2.9% in 2007, 6.9% in 2012). Although APT is characterized by a large public sector, employing almost 20% of the province's workforce, economic setting is characterized by small private firms: not only the average size is 3.7 employees, but around 90.0% of the firms has less than 10 employees, and more than two-fifths (43.5%) of employees work in firms with less than 10 employees. According to labor force statistics, services represent the cornerstone of economic development (about 53% of firms in 2010), followed by agriculture and tourism-related industries (23,24).

2. Occupational Injuries. Data on occupational injuries for all of APT from 2000 to 2013 were retrieved from the archive of the Operative Unit for Health and Safety in the Workplaces (UOPSAL, Italian acronym), including data from Emergency Departments, Public and Private Health Insurances, General Practitioners. UOPSAL is the institutional service representing the local governmental structure for the management and prevention of occupational injuries, occupational diseases, and work-related diseases in the workplaces. Retrieved data included any OEI defined as any injury occurring to the eye(s) and/or adnexa that occurred at the workplace, either as a part of the job, or on work-related assignment (25), whereas OEI occurring in (1) patients with major polytrauma, (2) students, (3) on route home to work place and vice-versa, were excluded from the study. Data were preventively

anonymized in order to include only: patients' age, gender, country of birth, injury diagnosis, body part included, product(s) involved, days of indemnity, and prognosis.

Data on work-force in APT were similarly obtained from the Statistical Institute of the Autonomous Province of Trento (ISPAT; http://www.statistica.provincia.tn.it/dati_online/), and included the number of active workers by age, sex, occupational groups, the latter being summarized for the categories: agriculture, manufacture and other industries, construction, and services.

2. Data Analyses. Injuries characteristics were initially summarized by means of descriptive statistics. Chi squared analyses were used to compare proportions for OEIs occurring in Agriculture vs. all other Occupational Groups. In the analyses, data were compared as follows: by gender (males vs. females); age groups (reference category: 25-39 years); migration background (Italian-born people vs. Foreign born people); day of the event (i.e. Monday to Friday vs. Weekend; Holiday vs. Regular day; April to September vs. October to March); reported hour of the events (06.00 to 12.00; 12.00 to 18.00; 18.00 to 06.00), with afternoon (12.00 to 18.00) assumed as the referent category; and characteristics of the OEI (i.e. associated with foreign objects; following eye contusions; eye lacerations; following exposure to chemicals, gas, and vapours; following exposure to other causes such as biological liquids, radiations, heat etc.), assuming OEIs by foreign objects as reference category.

Continuous variables were initially tested for normal distribution (D'Agostino & Pearson omnibus normality test): where the corresponding p value was < 0.10, normality distribution was assumed as rejected, and variables were compared through Mann-Whitney or Kruskal-Wallis test for multiple independent samples. On the other hand, variables passing the normality check (p value ≥ 0.10) were compared using the Student's t test or ANOVA, where appropriate.

Estimates of the numbers and rates (per 1,000 workers) of OEI were obtained by gender, age and occupational groups. As previously suggested (26,27), injury rates per 1,000 workers during the 2000-2013 period were calculated using the formula:

$$R=1{,}000 \times (I / N),$$

where I is the number of OEI claims over the 2000-2013 period and N equals the number of APT worker-years for all workers in the APT.

Injury rates by year were calculated using the formula:

$$Ry=1{,}000 \times (Iy / Ny),$$

where Iy is the number of OEI that occurred in a given year y and Ny equals the number of APT employees who worked in a given year y, y=2000, 2001, … 2013. Similarly, injury rates per 1,000 workers were obtained for gender, demographic groups (i.e. 15-24 years; 25-39 years; 40-54 years; 55-64 years; ≥65 years) and occupational groups.

Ninety-five per cent Confidence Intervals (95%CI) estimates were based on standard error estimates of R and Ry. Mantel-Haenszel combined estimates of the incidence-rate ratios (IRR) were then calculated for age and occupational groups by gender of the workers.

In order to assess the risk of OEI in Agricultural workers vs. all other Occupational groups, a multivariate analysis was eventually performed through a logistic regression analysis. The model included as outcome variable OEIs occurring in Agricultural workers compared to non-agricultural workers, and included all variables that had a p value <0.05 at univariate analysis, with calculation of respective Odds Ratios (OR) and correspondent 95% CI.

Significance level was p <0.05 for all statistical test. All analyses were conducted by using SPSS 24.0 (IBM Corp: Arkmon, NY).

3. Ethics. The study included only a retrospective assessment of data available through an Institutional Database, and the analysis was performed as a part of the official duties of the Occupational Health and Safety Unit (UOPSAL). Personal data were restricted to information about the OEIs, and were treated in order to guarantee the respect of privacy of the involved workers, as specifically stated by Italian Law n.674 of 1996 about personal data protection. Therefore, the study did not require preliminary evaluation by the local Ethical Committee.

Results

1. Demographics (Table 1). From 1 January 2000 to 31 December 2014, an estimated of 141,139 work-related injuries were recorded. Of them, 5065 (3.6%) were OEIs from 4802 workers, with an annual mean of 361.8 claims (range: 224-523; see Figure 1), 257 recurring cases (5.1% of all OEI injuries), and 160 episodes that eventually resulted in a RVL (3.2%). In around a third of RVL cases (1.3% of all events), reported binocular visual loss was higher than 4/20.

Overall, 91.9% of all cases occurred in males, of Italian origin (77.2%). OEI were most commonly seen in the age group of 25-39 year-old and 40-54 year-old years, that constitute 41.9% and 33.4% of all patients, respectively, whereas only 11.0% (n=457) of the cases occurred in workers older than 55 years. The mean age at injury was 38.4±11.7 years in all patients (range, 15 to 75), with 38.3±11.8 years in males, and 39.1±11.3 years in females, and no significant difference was found between males and females with regard to age at injury (p=0.230).

The industrial sector reported the higher share of OEI (70.7%), including 1487 cases (29.4%) from the construction sector, whereas 748 (14.8%) accounted to the agricultural settings. Overall, the majority of cases occurred Monday to Friday (70.9%), in winter months (October to March, 52.1%), during the afternoon (h 12:00 to h 18:00; 51.6%), with two peaks corresponding to hours 10:00 to 13:00 and 15:00 to 18:00 (Figure 2). Around half of total OEI cases were associated with a diagnosis of foreign body (50.2%), followed by eye contusions (21.8%), and lacerations (20.0%). Eventually, 6.8% of reported injuries followed the contact with chemicals, including irritating gases and vapours, whereas less than 1% was associated with the occupational exposure to heat and/or radiations (e.g. UV radiations, 0.7%), and biological fluids (0.3%).

The overall OEI rate was 1.66 / 1,000 person-year, 95%CI 1.45-1.87, being significantly greater in males (2.61/1,000 person-year, IC95% 2.30-2.93) than in females (0.35/1,000 person-year, IC95% 0.30-0.45; IRR 8.009 95%CI 7.244-8.684; p=0.0097) (Table 2).

As shown in Figure 1, even though a certain heterogeneity was reported over the assessed time frame, annual rates eventually decreased over time from

Table 1. Characteristics of 5065 Occupational Eye Injuries (OEI) occurring over the time period 2000 to 2013 in the Autonomous Province of Trento (APT).

	N/5065, %
Gender	
Males	4654, 91.9%
Females	411, 8.1%
Age group (years)	
15-24	694, 13.7%
25-39	2122, 41.9%
40-54	1692, 33.4%
55-64	486, 9.6%
≥65	71, 1.4%
Migration background	
No (Italian-born)	3912, 77.2%
Yes (Foreign-born)	1153, 22.8%
Working Sector	
Agriculture	748, 14.8%
Industry	3579, 70.7%
Construction	1487, 29.4%
Manufacture and other industries	2092, 41.3%
Services	738, 14.6%
Eye involvement	
Right	2490, 49.2%
Left	2544, 50.2%
Bilateral	31, 0.6%
Day of the week	
Monday to Friday	3591, 70.9%
Weekend	1474, 29.1%
Holidays	
Holiday	4982, 98.4%
Regular day	83. 1.6%
Calendar month	
April to September	2424, 47.9%
October to March	2641, 52.1%
Hour of the day	
Morning (06:00 to 12:00)	2148. 42.4%
Afternoon (12:00 to 18:00)	2613, 51.6%
Evening/Night (18:00 to 06:00	304, 6.0%
Diagnoses	
Foreign Bodies	2553, 50.4%
Contusions	1103, 21.8%
Lacerations	1013, 20.0%
Chemicals, gas, vapours, etc.	346, 6.8%
Heat, radiation (e.g. UV radiation, etc.)	33, 0.7%
Biological fluids	17, 0.3%
Outcome	
Residual visual loss, any	160, 3.2%
Residual visual loss > 4/20	68, 1.3%
Recurring events	257, 5.1%

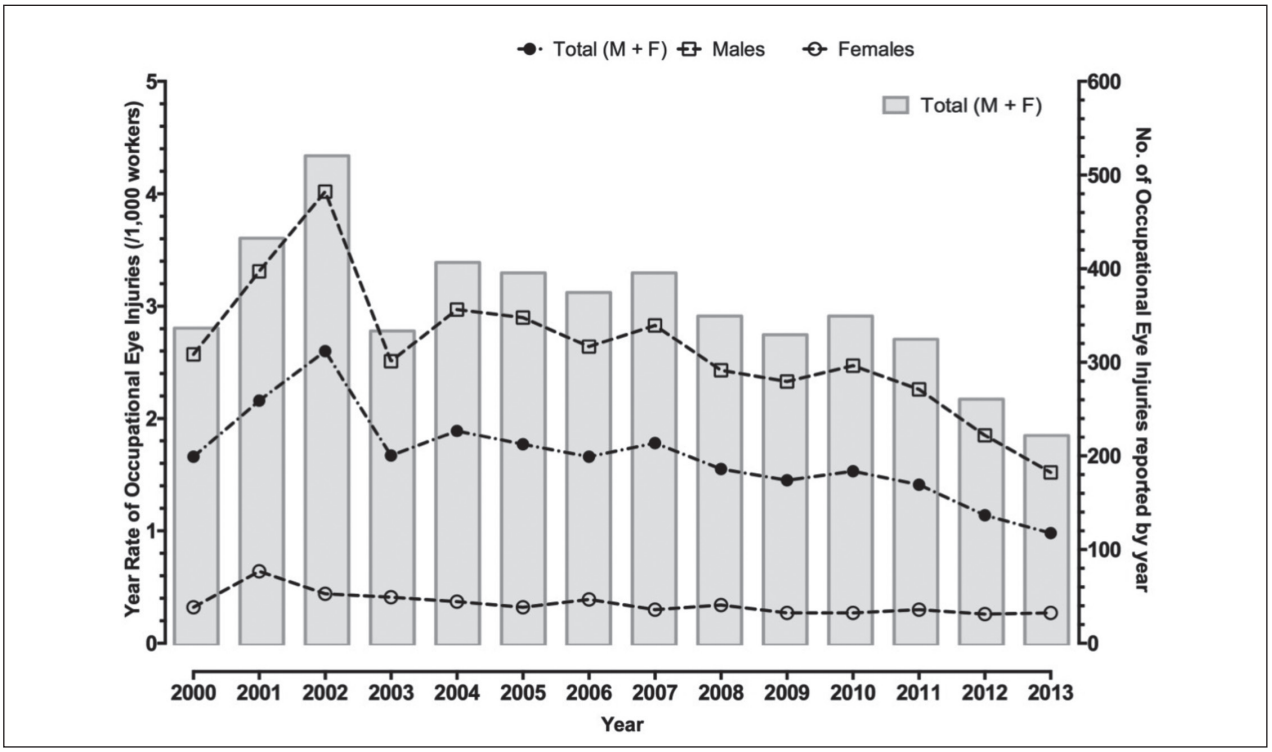

Figure 1. Yearly frequency of Occupational Eye Injuries (OEIs) over the assessed time period (2000 – 2013), both as incident cases (Males + Females; right y axis) and incidence rates (Males, Females, Males + Females; left y axis).

1.66/1,000 person-year in males and 0.32/1,000 person-year in females of 2000 to the lower rates of 2013 (1.52/1,000 person-year and 0.27/1,000 person-year, respectively). Also stratifying by age groups, the rates for OEI remained higher in males than in females for all age group: more specifically, males 15-24 years of age had the highest eye injury rate (4.44/1,000 person-year, 95%CI 3.67-5.21), whereas the lowest was found in females 40-54 years (0.29/1,000 person-year, 95%CI 0.24-0.33 person-year).

Focusing on the Occupational Groups, higher rates were reported from the Agricultural Settings (6.04/1,000 person-year, 95%CI 5.04-7.04), both for males (6.99/1,000 person-year, 95%CI 5.96-8.02) and females (2.26/1,000 person-year, 95%CI 1.49-3.03), and again the IRR was significantly greater for males than for females (3.373, 95%CI 2.490-4.377, p<0.001).

2. Characteristics of the Agricultural OEI. As shown in Table 3, 92.5% of OEIs from agricultural settings were reported in males, compared to 91.8% from other economic sectors, and the difference was not statistically significant (p=0.496). On the contrary, agricultural settings had a significantly higher share of OEIs from Italian-born people than other economic sectors (89.2% vs.75.2%, p<0.001), and the difference was significant also at multivariate analysis (OR 2.435, 95%CI 1.796-3.303).

Focusing on the age at the event, agricultural OEIs occurred in significantly older workers (mean age 45.6±13.3 years vs. 37.1±11.0 years in non-agricultural workers; p<0.001), and assuming 25-39 years group as the referent one, the association was significant for all older groups (OR 1.457, 95% 1.154-1.840 for age group 40-54 years; OR 3.749, 95% 2.819-4.985 for age group 55-64 years), and in particular for subjects older than 65 years (8.2% of all agricultural cases, vs. 0.3% in other occupational groups; OR 23.762, 95%CI 11.422-49.435), whereas younger subjects (15-24 year group) had a significantly lower risk (OR 0.579, 95% 0.388-0.863).

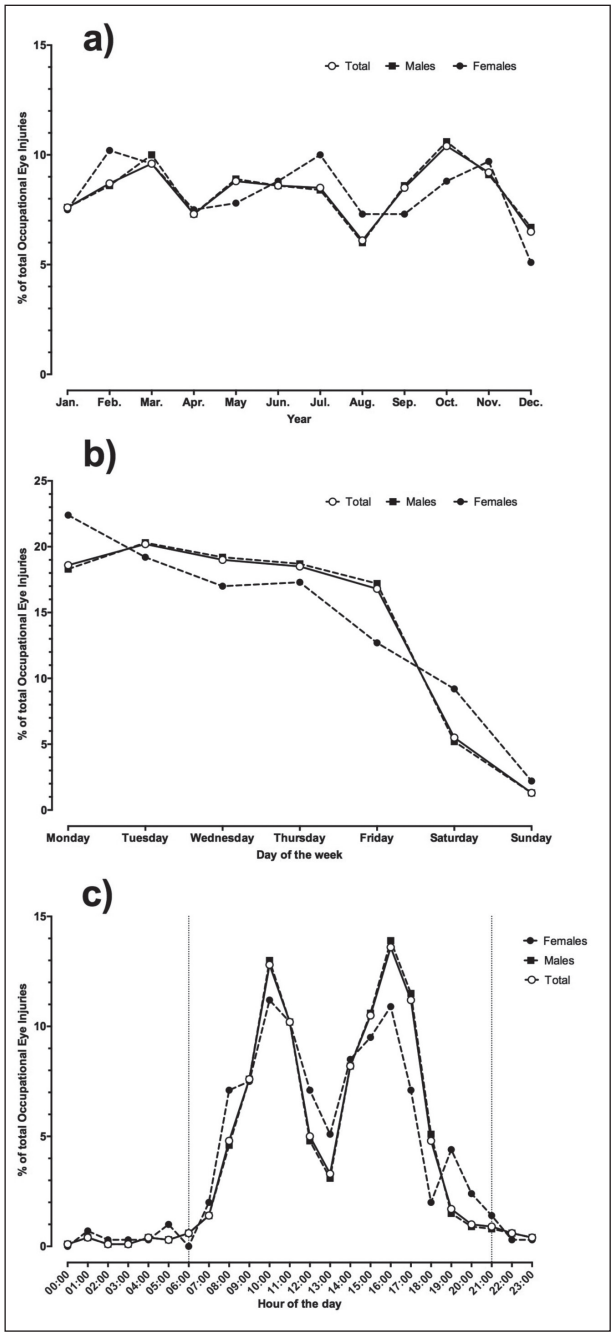

Figure 2. Share of incident Occupational Eye Injury (OEI) cases over the year (a), the week (b), and a one-day period (c)

Regarding the settings of the injury (Figure 2), no differences were found regarding the annual distribution, with similar share of events during winter months (44.8% vs. 48.4%, p=0.069), whereas OEIs in agriculture were more likely to be reported during the Week End (14.8% vs. 5.4%, p<0.001) and during Holidays (4.3% vs. 1.2%, p<0.001; OR 2.430, 95%CI 1.249-4.725) than in other occupational groups. Again, agricultural OEIs were more likely reported at morning (45.4% vs. 41.8%), and less frequently reported in the late hours of the day, at afternoon (50.6% vs. 51.8%), and in particular at night (4.0% vs. 6.4%) where-assuming the share of the afternoon OEIs as the referent category, an OR 0.510, 95%CI 0.312-0.836 was ultimately calculated.

Even though that of "foreign bodies" was the most frequently reported diagnosis both in agricultural and in non-agricultural OEIs, the shares were significantly different, being reported in 33.2% of all agricultural events and in 53.4% of all non-agricultural events (p<0.001). Assuming such diagnosis as the referent one, agricultural OEIs were more likely associated with "contusions" (OR 2.042, 95% 1.602-2.602) and "lacerations" (OR 2.386, 95%CI 1.877-3.033), and less frequently with exposures to chemicals, gases and vapours (OR 0.478, 95%CI 0.279-0.817) than non-agricultural OEI.

Eventually, when the final outcome of the OEI was put in consideration, agricultural injuries were apparently associated with a worse prognosis, as 5.9% resulted in a RVL vs. 2.7% in non-agricultural claims, and 2.1% of total agricultural OEI were followed by a RVL>4/20 compared to 1.2% in non-agricultural ones (p<0.001 and p=0.040, respectively). However, multivariate analysis did not confirm results of the univariate analysis (OR 1.175, 95%CI 0.667-2.070 for any RVL, and OR 1.068, 95%CI 0.449-2.450 for a RVL>4/20).

Discussion

OEIs are largely preventable, but their incidence remains strikingly high, not only in developing countries, but also in highly developed countries, with significant heterogeneity both in annual rates and in settings of the injuries (1,14,16). Since such differences have been explained as direct consequences of the work activities performed, also preventive measures have to be specifically adapted, underscoring the importance for all intervention that may improve our understand-

Table 2. Mean annual frequencies and respective 95% Confidence Intervals (95%CI) of Occupational Eye Injuries per 1,000 workers employed in the Autonomous Province of Trento by age groups and working sector, as broken down by sex, with calculation of respective Incidence Rate Ratios IRR)

	Mean Incidence (N/1,000 worker-years) (95%CI)			IRR (95%CI)	P value
	Total	Males	Females		
Overall	1.66 (1.45-1.87)	2.61 (2.30-2.93)	0.35 (0.30-0.40)	8.009 (7.237-8.864)	<0.0001
Age group (years)					
15-24	2.82 (2.32-3.32)	4.44 (3.67-5.21)	0.45 (0.31-0.58)	10.027 (7.383-13.939)	0.0097
25-39	1.60 (1.44-1.77)	2.54 (2.31-2.76)	0.30 (0.26-0.34)	8.748 (7.462-10.314)	
40-54	1.34 (1.18-1.49)	2.06 (1.84-2.28)	0.29 (0.24-0.33)	7.348 (6.200-8.746)	
55-64	1.66 (1.47-1.85)	2.31 (1.96-2.79)	0.34 (0.24-0.45)	6.571 (4.766-9.287)	
≥65	1.65 (1.09-2.21)	1.99 (1.20-2.79)	0.66 (0.29-1.03)	2.675 (1.228-6.911)	
Working Sector					
Agriculture	6.04 (5.04-7.04)	6.99 (5.96-8.02)	2.26 (1.49-3.03)	3.272 (2.490-4.377)	<0.0001
Construction	5.27 (4.65-5.89)	5.59 (4.99-6.19)	0.42 (0.14-0.69)	101.8 (51.530-236.2)	
Manufacture and other industries	3.85 (3.06-4.64)	4.58 (3.75-5.41)	0.96 (0.69-1.13)	5.421 (4.514-72.699)	
Services	0.36 (0.30-0.43)	0.53 (0.43-0.63)	0.23 (0.20-0.27)	2.283 (1.956-2.671)	

ing of the OEI epidemiology in all economic sectors, even at a local level (1, 4, 14).

In our study, OEI represented the 3.6% of all claims, with a relatively share of cases with RVL, <5% of all reported events. Such figures appear significantly lower to most other studies, suggesting rates up to 20% of all occupational claims, but it should be stressed that the rates significantly varies worldwide (3, 4, 10, 16, 19, 28, 29). Moreover, the cases we retrospectively evaluated were retrieved from an institutional database: despite we included several sources of information, it may fail to include minor injuries and illnesses, in particular those not requiring a medical treatment. On the contrary, hospital-based studied tend to underestimate the true impact of ocular trauma, biasing the results towards the more serious cases of ocular trauma (1, 15).

In other words, our figures are not fully comparable with previous reports from previous studies from Northern Italy, where data were retrieved from hospital department databases, and included as reference group all ophthalmological emergencies rather than occupational injuries and trauma as in our analysis (1, 14). On the other hand, frequency estimates appear substantially consistent with aforementioned reports: even though we estimated an incidence rate of 1.66/1,000 person-years, compared to 3/1,000 person-years reported by Gobba et al (2017) (14), and 6.5/1,000 person-years from Fea et al (2008) (1), our data refer to a 14-year time period, including annual rates that were quite similar to such estimates. As otherwise suggested, Italian figures should be interpreted as a consequence of the very large share of the total workforce involved in economic sector at low or very low risk for OEIs, such as that of services: economic data, suggest that during the assessed time-frame around 68% of all workforce of APT was actually employed in the tertiary sector, a share even higher than that reported by other Italian regions (e.g. around 50% in Emilia Romagna Region and 55% in Piemonte Region) (1,14,23,24). Not coincidentally, in our sample incidence estimates for OEI in services were 0.36/1,000 person-years (95%CI 0.30-0.43), that is 10 times lower than that for manufacture and other industries (3.85 1,000 person-years; 95%CI 3.06-4.64), the latter being usually reported as the highest risk groups alongside construction (1, 2, 11, 12, 3-10).

Similarly, specificities of both demographics and APT local economy may also explain some of the significant differences that emerge when our results are compared to other Italian data as well as to available international reports.

First at all, the large majority of our sample included workers of male sex, whose mean age was around 38 years. Male predominance is a common report from available studies, and may be attributed to

Table 3. Characteristic of occupational eye trauma occurring in agricultural workers compared to that reported from other economic sectors. Odds Ratios (OR) and respective 95% Confidence Intervals (95%CI) were calculated through a multivariate analysis that included demographic factors and characteristics of the event that, at univariate analysis, had a p value < 0.05.

	Agriculture N/748, %	Other Economic Sectors N/4317, %	Chi squared P value	OR (95%CI)
Gender				
Males	692, 92.5%	3962, 91.8%	0.496	-
Females	56, 7.5%	355, 8.2%		-
Age group (years)				
15-24	44, 5.9%	648, 15.0%	<0.001	0.579 (0.388; 0.863)
25-39	212, 28.3%	1908, 44.2%		-
40-54	269, 35.9%	1420, 32.9%		1.457 (1.154; 1.840)
55-64	162, 21.7%	328, 7.6%		3.749 (2.819; 4.985)
≥ 65	61, 8.2%	13, 0.3%		23.762 (11.422; 49.435)
Migration background				
Italian-born	667, 89.2%	3245, 75.2%	<0.001	2.435 (1.796; 3.303)
Foreign-born	81, 10.8%	1072, 24.8%		-
Eye involvement				
Right	356, 47.6%	2134, 49.4%	0.249	-
Left	390, 52.1%	2154, 49.9%		-
Bilateral	2, 0.3%	29, 0.7%		-
Day of the week				
Monday to Friday	637, 85.2%	4082, 94.6%	<0.001	0.364 (0.260; 0.511)
Weekend	111, 14.8%	235, 5.4%		-
Holiday	32, 4.3%	51, 1.2%	<0.001	2.430 (1.249; 4.725)
Calendar month				
April to September	335, 44.8%	2089, 48.4%	0.069	-
October to March	413, 55.2%	2228, 51.6%		-
Hour of the day				
06:00 to 12:00	340, 45.4%	1805, 41.8%	0.048	0.960 (0.787; 1.171)
12:00 to 18:00	378, 50.6%	2236, 51.8%		-
18:00 to 06:00	30, 4.0%	276, 6.4%		0.510 (0.312; 0.836)
Diagnoses				
Foreign Bodies	248, 33.2%	2305, 53.4%	<0.001	-
Contusions	232, 31.0%	871, 20.2%		2.042 (1.602; 2.602)
Lacerations	245, 32.8%	768, 17.8%		2.386 (1.877; 3.033)
Chemicals, gas, vapours, etc	22, 2.9%	324, 7.5%		0.478 (0.279; 0.817)
Other	1, 0.1%	49, 1.1%		0.175 (0.024; 1.307)
Outcome				
Residual visual loss, any	44, 5.9%	116, 2.7%	<0.001	1.175 (0.667; 2.070)
Residual visual loss >4/20	16, 2.1%	52, 1.2%	0.040	1.068 (0.449; 2.540)
Recurring events	65, 8.7%	292, 6.8%	0.057	-

the traditional occupational differences, with a disproportionate number of males working in occupations with a higher risk for ocular trauma (1, 2, 11, 12, 14, 15, 18, 20, 3-10).

Despite such data are somehow consistent with certain reports, suggesting higher risk for male subjects between 25 and 44 years of age (9, 18, 20), mean age remains relatively lower than that otherwise reported by Gobba et al (2017), and relatively high compared to other studies from comparable settings (1, 2, 11, 12, 14, 15, 3-10). Again, even though higher rates were reported from younger workers (2.82/1,000 person-years for 15-24 years age group), consistently with the evidence of higher OEI risk for workers <40 year-old, rates remained substantially stable over the various age groups. Such differences may be explained as our esti-

mates included only occupational injuries, and demographic figures from other Italian studies are therefore only limitedly comparable (1, 14, 15).

On the contrary, the specific characteristics of the recorded events are somewhat comparable with available reports. First at all, the main cause of OEI was identified in foreign bodies (50.4%), followed by contusions and lacerations. Also in previous reports from Northern Italy, the large majority of injuries were due to a trauma (around 90% of all occupational injuries), being associated with foreign body in nearly half of reported cases (1, 14). This was not unexpected, as the occupational injuries usually reflect the economic and industrial development of the referent population (4, 10, 11, 19). For instance, while OEIs associated with chemicals, gas, vapours, but also with tasks usually reported in the heavy industry such as welding, drilling and cutting, are relatively rare in our study, in Zakrewski et al (2017) chemical exposure and subsequent chemical lesions of the cornea were among the main aetiologies of injury (4), whereas in Chen et al (2009) welding was a common cause of a work-related eye injury (30.4%), as well as the splashing of chemicals (11.7%), being photokeratitis (33.2%), and lesions characterized by chemical burns and corneal abrasions (collectively 26.5%) more frequently reported than eyeball penetration/laceration (22.3%) (10). Also in Yu et al study (2003), still reporting flying objects (59.4%) as the main causes of OEIs, chemical substances (18.4%), and hazardous radiations (2.5%) were significantly more frequent than in our report (11).

Focusing on the specificities of agricultural OEIs, events occurred more frequently in subjects of Italian origin and older age groups. Moreover, agricultural OEIs exhibited several specificities in terms of clinical characteristics, with higher representation of trauma and laceration over foreign bodies, and regarding settings of the events. Such remarks may be seemly explained as a consequence of the specificities of the agricultural activities.

First at all, there is sound evidence that primary sector employs older from older age groups, as more than half of farm managers across EU are usually older than 55 years, being close or beyond the regular retirement age (30). Reflecting the demography of rural population, patients reporting OEIs in agricultural settings are therefore usually older than those from other economic sectors (31).

Moreover, in Trentino region, a large share of farm managers performs agricultural activities as hobby farmers, in holding of small extent (23, 24), eventually explaining the relatively low share of events recorded in subjects from a migrant background. Such remarks contribute to the interpretation of the specific time-frame of the OEIs in agricultural settings. On the one hand, it should be stressed that many agricultural activities are not always very flexible, having to be performed in a restricted time window, not allowing the agricultural workers to usually spare holidays and weekend (23). On the other hand, as many agricultural workers are actually hobby farmers, a significant share of their tasks cannot be performed during the working week, being forcedly clustered during weekend or holydays (23, 24, 30).

Focusing on the reported diagnoses, OEIs in agricultural settings exhibited higher share of trauma-related event, with a significantly lower risk for injuries following the exposure to chemicals, gas and vapors, the latter being even lower than in other similar reports (14). Actually, agricultural workers are exposed to strenuous physical activities, therefore explaining a higher proportion for blunt trauma (23, 24, 30, 32). On the other hand, available reports about the use of pesticides and chemicals in Trentino region suggest a diffuse understanding of appropriate preventive practices during pesticide handling, including the proper use of PPE during tasks at higher risks (33, 34).

Unfortunately, the main limit of our study may be found in the lack of information about the use of PPE at the time of the event. Nearly all available reports consistently associate the inappropriate use of PPE with OEI, with shares of patients who did not wear any PPE at the time of injury ranging from 66.9% to 85.4% (4, 10, 11, 14, 35). More specifically, Bureau of Labour Statistics of US estimates that as many as 60% of workers within the US who sustain an occupational eye injury had failed to use eye PPE (9, 26, 28).

In conclusion, our results show a relatively lower frequency of OEIs compared to other studies previously published, with a relatively lower proportion of RVL. In our study, agricultural activities were associated with a seemly higher incidence than that reported in

other occupational groups. OEIs in agricultural workers exhibited a specific pattern, both in terms of lesion, and settings of the events, recommending tailored interventions in order to improve promotion strategies. More specifically, even though our study lack data about the use of PPE at the time of the event, our data speculatively suggest the advocacy for improved use and appropriate selection of eye PPE not only during activities requiring the handling of chemicals or irritating substances, but also during strenuous physical activities potentially eliciting blunt trauma of the eye.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Correlations between tumor-infiltrating and circulating lymphocyte subpopulations in advanced renal cancer patients treated with nivolumab

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Summary. *Background:* In clinical trials with immunotherapy, histological features such as tumor-infiltrating lymphocytes (TILs) are investigated as potential predictive biomarkers, with the limit of an outdated parameter for a typically dynamic element. *Methods:* This explorative study compared, in metastatic renal cell carcinoma (mRCC) patients, basal pathological data about TILs on diagnostic histological specimens with circulating lymphocyte subpopulations measured before and during therapy with nivolumab. *Results:* Of 11 mRCC patients, 5 had low presence of TILs (L-TILs), 3 moderate amount (M-TILs) and 3 high number (H-TILs). Overall, 8 patients had low intratumoral pathological CD4+/CD8+ ratio (LIPR) ≤1 and 3 cases high intratumoral pathological ratio (HIPR) ≥2. Of 8 patients with LIPR, only 2 matched with low circulating CD4+/CD8+ ratio (LCR) ≤1; 5 had high circulating ratio (HCR) ≥2. All 3 cases with HIPR (≥2) conversely had LCR (≤1). Circulating CD4+/CD8+ ratio remained unchanged during therapy (mean -0.12 in 8 weeks). The respective percentage values of CD4+ and CD8+ circulating T cells also remained stable (variation 0%); the absolute value of CD4+ was more likely to increase (mean +46.3/mm^3); the level of CD8+ tended to slightly decrease (mean -6.5/mm^3). No correlation of lymphocyte subpopulations with treatment outcome was found. Of note, we did not evidence correspondence between histopathological and circulating findings in terms of T-lymphocyte subpopulations, also suggesting the inconsistency of circulating data in terms of relative variations. *Conclusions:* Considering the likely high dynamism of TILs, rebiopsy before therapy might be proposed to assess the utility of TILs characterization for predictive purpose. (www.actabiomedica.it)

Key words: tumor infiltrating lymphocytes, renal cell carcinoma, circulating lymphocytes, immune checkpoint inhibitors, nivolumab

Background

The characterization of the tumor microenvironment is progressively acquiring the right crucial role for the strategy of harnessing the immune system to fight cancer. The mechanisms of immune escape are finally investigated, identified and described as strongly related to this complex and dynamic element (1).

One of the approaches attempted for the tumor microenvironment investigations is constituted by the characterization of tumor infiltrating lymphocytes (TILs), a possible manifestation of antitumor immunity. As a rule, the prognostic significance of abundant TILs has a positive connotation, representing the manifestation of antitumor immunity (particularly CD8+ T cells), as historically known and extensively

demonstrated for melanoma, breast and lung cancer (2-4).

Nevertheless, previous data in human renal cell carcinoma (RCC) suggest that infiltration of tumor tissue by T cells itself does not denote the efficacy of antitumor immunity, because it may be related to the the biological malignancy of tumor cells.

From a clinicopathological analysis of the biological significance of TILs in 221 cases of surgically resected RCC, Nakano et al demonstrated a correlation between abundant infiltration of tumor tissue, not only by CD8+ but also by CD4+ T cells, and a shorter survival of the patients. It was due to a positive correlation between the number of lymphocytes and representative tumor grade factors, thus suggesting that immune cell reactions are more pronounced as the tumor biological malignancy progresses, probably because of increased antigenicity of tumor cells (5).

Some features of TILs in terms of quality and quantity (immunohistochemistry with count and subpopulations) have been related to prognostic or predictive characteristics. For instance, in renal cell carcinoma (RCC) high density CD4+ T-cell infiltrate is associated with unfavorable tumor characteristics and poor prognosis (6-7). Moreover, also the relative ratios of the various TILs subpopulations deserve to be evaluated.

Only few studies correlated the basal features of TILs with the dynamic circulating T cells counterpart.

Such comparison was performed by Asma et al on CD4+ regulatory T cells (T-reg), which intratumoral and circulating subpopulations have been respectively investigated, with the further interesting comparison of circulating T-reg of healty donors (8). The investigators demonstrated that the proportion of T-reg in TILs was, in average, like that found in circulating CD4+ T cells of patients or healthy donors.

A similar but more detailed investigation was conducted in a very recent study by Giraldo et al, providing a multiparametric flow cytometric immunophenotypic analysis of TILs (defined as T cells isolated from tumor tissue), of T cells adjacent non-malignant renal tissue (defined as renal-infiltrating lymphocytes, RILs) and of peripheral blood lymphocytes (PBL), in a cohort of 40 patients with localized RCC (9). On the basis of TILs phenotypic characterization, they identified three dominant immune profiles in localized RCC, respectively called “immune-regulated” in inflamed tumors (22%), characterized by polyclonal/poorly cytotoxic CD8+/PD-1+/Tim-3+/Lag-3+ TILs and CD4+/ICOS+ cells with a T-reg phenotype (CD25+/CD127-/Foxp3+/Helios+/GITR+), highly PD-L1 positive; “immune activated” (22%) enriched in oligoclonal/cytotoxic CD8+/PD-1+/Tim-3+ TILs; and “immune silent” (56%), enriched in TILs exhibiting RIL-like phenotype. Only immune-regulated tumors resulted to have aggressive histologic features, high risk of disease progression after nephrectomy and a CD8+/PD-1+/Tim-3+ and CD4+/ICOS+ PBL phenotypic signature. According to the results of this study, in localized RCC, the infiltration with CD8+/PD-1+/Tim-3+/Lag-3+ exhausted TILs and ICOS+ T-reg identifies the patients with poor prognosis who could potentially benefit from adjuvant therapy with checkpoint blockade (9).

In clinical trials with immune-checkpoint inhibitors (CKI) in metastatic RCC (mRCC), histological features such as TILs in the primary tumor are investigated as potential predictive biomarkers, with the possible limit of an outdated parameter for a typically dynamic element.

Up today, no studies with the paired analysis of tumor infiltrating and circulating lymphocytes subpopulations in renal cancer patients in relation to the new immunotherapy with CKI have been published and any biomarker has been identified to predict response to nivolumab in such population.

Materials and methods

This explorative study compared, in consecutive mRCC patients treated with nivolumab, basal pathological data about TILs on diagnostic histological specimens from nephrectomies with circulating lymphocyte subpopulations, evaluated before and during therapy, considering both quantitative and qualitative features, with the primary aim to assess their concordance. Furthermore, a possible correlation of such features with treatment outcome was explored, assessing the treatment outcome in terms of objective responses through the complete, blinded radiologic revision of

basal computed tomography (CT) scans and of at least two subsequent CT scans assessment during treatment with nivolumab. The revision was performed by three radiologists, with at least two of them independently revising each exam, according both to the standard RECIST 1.1 and to the immune-related RECIST (iRECIST) (10-11).

For the pathologic analysis, we assessed at least 3 slides with well-preserved neoplastic tissue per each tumor sample. The slides were stained with hematoxylin and eosin for conventional evaluation, too. For the assessment of TILs, of CD8+ and CD4+ cells, we considered both qualitative and quantitative parameter. The presence of TILs was evaluated as "absent", "low", "moderate", or "high", with both intratumoral and peritumoral assessment. The presence of CD4+ and CD8+ cells and CD4+/CD8+ ratio was also evaluated in both sites. Immuno-histochemical staining of formalin fixed, paraffin-embedded, tissue sections was performed on all samples. After deparaffinization and rehydratation sections were treated with 3% hydrogen peroxidase for 5 mins. For antigen retrieval, sections were treated with pH9 Tris-EDTA buffer for 30 mins in water-bath at 98°C. Sections were stained with the following primary antibodies: CD4 (Roche; diluition ready to use), CD8 (Roche; diluition ready to use). The sections were immunostained with a polymeric system Ultraview DaB Detection Kit (Ventana-Roche) in accordance with the manufacture›s specifications. Diaminobenzidine (DAB) was used for staining development and the sections were counterstained with haematoxylin. Negative controls consisted of substituting normal serum for the primary antibody.

Results

Eleven mRCC patients consecutively treated with nivolumab in second or subsequent lines at our institution were included in this analysis. Table 1 shows patients' characteristics.

Our histopathological results demonstrated a heterogeneous expression of TILs, including CD4+ and CD8+ T cells. The heterogeneity was both inter-patient and intra-patient, with several cases of discordant CD4+/CD8+ ratio and different presence of immune infiltration among peritumoral and intratumoral tissue.

Of 11 patients included, the intratumoral analysis identified 5 cases with low presence of TILs, 2 with

Table 1

Sex	
male	7 (64%)
female	4 (36%)
Age	
mean	80
range	56 83
Histology	
clear-cell carcinoma	5 (45%)
mixed (clear/non-clear)	3 (27%)
chromophobe	2 (18%)
papillary	1 (10%)
ECOG PS	
0	9 (82%)
1	2 (18%)
≥2	0 (0%)
MSKCC risk group	
poor	0 (0%)
intermediate	7 (64%)
good	4 (36%)
IMDC risk group	
poor	0 (0%)
intermediate	6 (64%)
good	4 (36%)
Treatment line with Nivolumab	
second	7 (58%)
third2 (18%)	
further lines (4th or subsequent)	2 (17%)
Previous treatment received (immediately before Nivolumab)	
Pazopanib	3 (27%)
Sunitinib	5 (45%)
Everolimus	1 (9%)
Axitinib	1 (9%)
Sorafenib	1 (9%)
Nephrectomy	
yes	10 (91%)
no	1 (9%)

PS=performance status; MSKCC=Memorian Sloan Kettering Cancer Center; IMDC=International Metastatic Renal Cell Carinoma Database Consortium

moderate amount and 4 with high number of lymphocytes.

Overall, 9 patients had low intratumoral pathological CD4+/CD8+ ratio (≤1) and 2 cases had high intratumoral pathological CD4+/CD8+ ratio (≥2). Of 9 patients with the low pathological ratio, only 3 cases matched with low circulating CD4+/CD8+ ratio (≤1), whilst 5 cases had high circulating CD4+/CD8+ ratio (≥2). One case was undetermined (not evaluable). The 2 cases with high pathological ratio (≥2) conversely had low circulating ratio (≤1).

Unexpectedly, independently from the clinical outcome, circulating CD4+/CD8+ ratio remained unchanged during therapy with CKI in each patient, maintaining about the same value after 8 weeks (mean +0.18). The respective percentage values of CD4+ and CD8+ circulating T cells also remained stable during treatment (mean variation 0%); the absolute value of CD4+ was more likely to increase (mean gain +100.6/mm3); the level of CD8+ tended to decrease (mean loss -30.6/mm3).

Interestingly, several cases (3) of opposite findings among intratumoral and peritumoral lymphocytes presence and differences in terms of CD4+/CD8+ ratio were found (Table 2).

With the limit of a small sample size, no clear correlation of either tissue or circulating lymphocyte subpopulations with treatment outcome was found. In our group, only one patient had partial response; the disease control rate was 45%; 6 patients (the majority, 54.5%) were primary refractory to nivolumab, confirmed at the second radiologic assessment during therapy. Interestingly, a complete concordance of responses was found between RECIST 1.1. and iRECIST in these 11 cases, with the limit of having stopped such radiologic comparison at the second CT scan after the basal one.

Discussion

Before sinking into the exquisite scientific digressions on the T cell-mediated immune microenvironment emerged by our study, we should disclose that some particular features, mainly due to a selection bias from retrospective nature and small sample size, emerged in this population and should be considered to not misinterpret our findings.

First, the mean age of our population was of 80 years, with a range of 56-83. Despite not completely

Table 2

Case number	Intratumoral TILs	Intratumoral CD4+/CD8+ ratio	Peritumoral-infiltrating lymphocytes	Peritumoral CD4+/CD8+ ratio	Baseline circulating CD4+/CD8+ ratio	Circulating CD4+/CD8+ ratio after therapy	Treatment response according to irRECIST
Case N. 6954	Low	1:2	Low	1:3	2.5	3	PD (progression of disease)
Case N. 8205	High	1:3	High	3:1	3.5	4.9	PR (Partial response)
Case N. 10622	Low	2:1	High	1:2	1	0.9	SD (stable disease)
Case N. 15859	Moderate	1:2	Moderate	1:2	0.9	NE (not evaluated)	PD
Case N. 18659	Low	1:2	High	1:2	1.1	1.1	PD
Case N. 7985	Low	only CD8+	Moderate	1:2	2.7	2.2	SD
Case N. 2011	High	1:3	Moderate	1:2	NE	NE	PD
Case N. 274	Moderate	1:3	Moderate	1:2	2.8	2.4	PD
Case N. 5007	High	1:2	High	1:2	0.5	0.4	PD
Case N. 552	High	1:2	High	1:3	2.5	3.8	SD
Case N. 25620	Low	2:1	Low	1:2	0.3	0.4	SD

representing the elderly, this element should be taken in account because of the possible "exhausting effect" of the age on the immune system. Concerning the treatment outcome, a lesser degree of benefit in terms of overall survival was seen for the elderly in the pivotal trial CheckMate025, particularly among patients aged 75 years, with a trend that appeared to favor the comparator used in the control arm (12). This evidence could provide a justification for the scarce outcome achieved in our group (best responses according to immune-related RECIST are shown in Table 2).

Second, much of cases had non-clear cell histology (55%): it is still largely unknown if the microenvironment of clear-cell RCC differs from those of rarer histologies such as papillary, chromophobe or mixed tumors, all represented in our sample. Further, the same type of immune microenvironment might acquire different meaning and opposite prognostic value in cases with different histologic subtype. Up today, no data on non-clear cell histologies treated with CKI have been reported, confounding the interpretation of the present study also concerning the treatment outcome.

Third, 64% of patients were classified as intermediate risk both per MSKCC and IMDC criteria, increasing the "average risk level" of the entire population, with an unavoidable negative prognostic impact.

These unusual aspects should be considered, suggesting a reflection about the possibility that response to immunotherapy can mainly depend on age and clinical conditions.

The most unexpected but also the most interesting findings of our analysis are represented by three key concepts: the lack of correlation between tissue and circulating lymphocyte subpopulations; the opposite findings among intratumoral and peritumoral T cell infiltrate; the lack of variation of the circulating CD4+/CD8+ ratio during therapy. A further gold concept, here confirmed but already well known by the literature concerning this cancer type (13), is represented by the inter-patient and intra-patient heterogeneity.

Analyzing the cited issues, the first key element of the lack of correlation between tissue and circulating lymphocyte subpopulations may confirm that the treatment choice should not be based on the evaluation of the immune microenvironment of the primary tumor, as instead it was done in clinical trials with CKI, selecting or stratifying patients basing on the primary programmed-death ligand 1 (PD-L1) expression. Our experience once again suggests to carefully consider the risk of basing the patients' selection for immunotherapy trials on an outdated parameter (the primary tumor analysis) for the evaluation of a typically dynamic element (the immune microenvironment).

The specular results in some cases obtained when comparing intratumoral and peritumoral T cell subpopulations might cast the doubt that the same T cell population plays a different role in the two sites and that, conversely, the same role is played respectively in the two compartments by two different T cell types. Not surprisingly in the light of our current results, in the already cited study by Asma et al, despite a similar average proportion of T-reg in circulating and infiltrating component from a quantitative point of view, intratumoral T-reg exhibited a marked different phenotype when compared with the autologous circulating T-reg. Intratumoral T-reg showed a higher inhibitory function on autologous CD4+/CD25- T cells when compared with circulating T-reg. Despite comparing different districts, in this case the same T cell plays different roles based on the site where it is located.

Eventually, about the unexpected lack of variation of the circulating CD4+/CD8+ ratio during therapy, the reason in our case could be provided by the possible immunosenescence of our population. Immunosenescence is generally defined as age-related changes to the immune system that result in increased susceptibility to infectious diseases and a decreased response to vaccination. Several demonstrations of this issue have been reported, among others the evidence that the proliferative response of T cells from older adults after vaccination against hepatitis B is diminished, or the age-related decline in toll like receptor-induced expression of the CD80 costimulatory molecule that is associated with a decrease in humoral immunity to influenza vaccine; or moreover the decline in the frequency of influenza-specific CD4+ memory T-cells, and the decreased cytolytic properties CD8+ effector and effector memory cells in response to influenza vaccine in older subjects (14).

Despite the general expectancy of a better outcome in the case of CD8+ abundance and of a possible

negative prognostic impact of the CD4+ prevalence, in our population neither CD4+ nor CD8+ prevalence in any site did not seem to have a predictive role (the peritumoral/intratumoral pattern and the ratio were heterogeneous both in responders and refractory cases). The role of cytotoxic CD8+ TILs in the setting of RCC is still controversial, with conflicting results according to different studies (5, 9, 15-18), maybe due to technical factors, including antibodies and techniques used to detect CD8+ T-cells (immunohistochemistry versus flow cytometry) and tumor site examined (peritumoral versus intratumoral TILs). Of most relevance was the ability of previous studies to differentiate between the effector cytotoxic CD8+ T cells and their exhausted counterparts, expressing high levels of immune checkpoint molecules in a dysfunctional immune environment. When dichotomized in such a way, Giraldo et al clearly demonstrated good prognosis with the former CD8+ T-cell population, and poor prognosis with the latter (9). Similarly, Nakano et al showed that TILs with high CD8+ T-cell content that exhibited high proliferative activity were associated with improved survival among patients with mRCC (5). Unfortunately, we were unable to distinguish between these two T-cell subpopulations.

Concerning CD4+ component, despite the well-known prevalence of the role CD8+ effector T cells in the antitumor immunity, recent evidence also demonstrated the existence of CD4+ T-cell-mediated antitumor immunity, with signatures of heterogeneous T-cell expansion in CD4+ TILs repertoire (19). In our sample, the previously discussed negative prognostic impact of CD4+ TILs abundance did not emerge.

A further element to be considered is represented by previous treatments, which undoubtedly modulated the immune microenvironment, potentially becoming responsible of a different subsequent outcome of immunotherapy. For instance, considering the two standard tyrosine kinase inhibitors used in first line setting for mRCC treatment, it has been demonstrated that pazopanib appears to function as a potent activator of dendritic cells in vitro and in vivo associated with the neo-activation of a CD137+ T cell population (20), whilst sunitinib seems to promote TILs expansion by reducing intratumoral content of myeloid-derived suppressor cells (21).

In our population, previous drugs were collected (see Table 1) but no relationship of this element has been found with the T cell immune pattern or TILs presence, despite the trend of a more favorable clinical outcome for pazopanib-pretreated patients (the only partial response received prior pazopanib and any patient pretreated with this drug was primary refractory).

The main limit of our analysis is represented by the small sample size. Another limitation is represented by the missed identification of T-reg, maybe resulting in masking the real prognostic or predictive effect of the other respective subpopulations.

Considering these important limits, this study did not evidence significant correspondence between histopathological and circulating findings in terms of T-lymphocyte subpopulations in mRCC patients undergoing treatment with CKI, also suggesting the inconsistency of circulating data in terms of relative variations. Moreover, heterogeneity concerning immune infiltrate and the often opposite results for intratumoral and peritumoral subpopulations can invalidate the research of a clear relationship between pathological and circulating data.

Considering the likely high dynamism of TILs, rebiopsy before CKI therapy might be the most reliable way to assess the utility of TILs characterization for predictive purpose.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Reducing complications in post-bariatric plastic surgery: our experience and literature review

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Summary. *Background:* With the rise in obesity, there has been a similar increase in bariatric surgery. This resulted in numerous patients losing significant weight with accompanying circumferential body contouring issues. This has led to an amazing increase in the number of body contouring procedures performed. *Methods:* The aim of this work is to revise the cases of body contouring in 78 ex-obese patients who underwent body contouring surgery in the Department of Health Life and Environmental Sciences – Plastic Reconstructive and Aesthetic Plastic Surgery Section, from 2007 to 2016. *Results:* The authors have noticed a deep relationship between adverse events and cigarette smoking and with pre-operative BMI. Regardless of these variables, the authors focused on the protocol for the management of patients, which required a collaboration between medical and nursing staff. *Conclusions:* Ex-obese patients have an important risk for complications, but the comparison of our personal data with those of the international literature confirms the efficacy of our management protocol with regard to the prevention of complications. (www.actabiomedica.it)

Key words: obesity, body contouring surgery, prevention of complications, nursing management, treatment protocol

Introduction

At the other end of the malnutrition scale, obesity is a major health problem emerging today, but yet very underestimated. It is, in fact, a major risk factor for a number of chronic diseases. Bariatric surgery is indicated for morbidly obese patients (BMI ≥ 40) who fail to respond to dietary, behavioral, nutritional, and medical therapies (1, 2). Weight-loss operations may cause restriction of food intake, malabsorption, or a combination of the two. Given the scarcity of data that compare the different bariatric operations, it remains impossible to make definitive guidelines for one procedure over another (3-6). With the increasing number of weight loss surgery, there is a higher number of patients who desire a body contouring surgery, since that the weight loss often results in a cutaneous excess that invalidates patients in their daily life: it causes mechanical limitation of physical activities, hygienic problems, infections, intertrigo or maceration. The goal of post-bariatric body contouring is to ameliorate the discomfort due to the bariatric surgery with removal of the excess tissue (7) and it should become a routine part of the multi-disciplinary approach for these patients. Post-bariatric plastic surgery is recommended for patients with a BMI <35 Kg/m2, a weight loss >30 Kg and stable weight for 6 months to 1 year, in good nutritional conditions and good health (8-10).

Materials and methods

The aim of the study was to revise the cases of body contouring in the ex-obese patients. We first col-

lected the data of patients who underwent bariatric surgery between 2007 and 2016 in our reference private hospital (Casa di Cura Privata "Di Lorenzo srl" - Avezzano, AQ) (11). Then, we analyzed how many of these patients presented for plastic surgical correction of contour abnormalities. From 2007 to 2016, 307 patients underwent bariatric surgery in the Operative Unit of General Surgery. Among these, 186 (60.6%) patients underwent gastric bypass, 77 (25.1%) patients underwent gastric banding and 44 (14.3%) patients underwent sleeve gastric resection. All these operations were performed laparoscopically by the same surgeon.

Of the 307 patients, 78 (25.4 %) underwent body contouring surgery in the same hospital with a time interval of at least 2 years following the bariatric procedure. In particular, the distribution of bariatric surgical procedures among these 78 patients was as follows: 44 (56.4%) gastric bypasses, 20 (25.6%) gastric bandings, 14 (17.9%) sleeve gastric resections. The following data and variables were collected: patient's age, sex, BMI at the time of body contouring surgery, the type of body contouring procedure, smoking habits, complications and interventions associated with each complication. Surgical procedures that we have considered in our study included: abdominoplasty, vertical thigh lift, brachioplasty, breast lift with implants and complete body contouring. The occurrence of adverse events was obtained and associated with the smoking habit and with the BMI at the time of body contouring surgery. We have considered the medical and nursing management and treatment of patients in the various stages (pre-, intra- and post-operative), according to a protocol that was developed by our research group and that has been validated by the ethics committee of the hospital used as a reference. The protocol we have used is shown in Table 1. We have categorized our outcomes into 4 grades (Excellent, Good, Moderate and Poor), according to the following criteria:

- Clinical outcome
- Visual Analog Scale for Pain (VAS Pain) (12)
- Patient satisfaction
- Presence and type of complications.

A - Clinical outcomes, evaluated six months after surgery: we have taken into account symmetry of the scar, positioning of the scar, harmony of the body profile, and we expressed a rating on a scale from 1 (poor) to 10 (excellent).

B - Visual Analog Scale for Pain (VAS Pain), administered at the time of hospital discharge: from "no pain" (score of 0) to "worst imaginable pain" (score of 10).

C - Patient satisfaction: the Moorehead-Ardelt Quality of Life Questionnaire II (13). Six key areas were examined: 1) general selfesteem, 2) physical activity, 3) social contacts, 4) satisfaction concerning work, 5) pleasure related to sexuality, and 6) focus on eating behavior. Each of these questions offered 10 possible answers, from 1 (poor) to 10 (excellent). The Questionnaire was administered on the day of surgery and six months after surgery. We compared the average value of the first questionnaire with the average value of the second, to evaluate the increase in the average score (from 0 to 10).

D - Complications, assessed at 6 months: their presence or absence, if it was simply an outpatient treatment or if it required a surgical treatment.

We have established for each of the criteria the thresholds and the overall evaluation was based on the following rule:

"Excellent" if:
A> = 8
B<=4
C>=1,5
D: no complications

"Good" if:
A >=6
B<=5
C>=1
D: if there was a complication, it did not require a surgical treatment

"Moderate" if:
A>=5
B<=8
C>= 0.5
D: no complications or presence of any type of complication

"Poor" if:
all the thresholds of "moderate" were not reached.

Table 1. Medical and nursing management and treatment protocol

1. Pre-operative stage: - Medical history with attention to comorbidities and drugs (steroids, contraceptives, antihypertensives, anticoagulants, hypoglycemic agents, antibiotics, sedatives, stimulants) - Check of the BMI and of any changes in the last 6-12 months - Blood chemistry and instrumental screening - Evaluation of diet and / or nutritional deficiency - Visit by the anesthetist - Psychiatric / psychological counseling - Suspension of drugs containing acetylsalicylic acid 30 days prior to surgery - Abstention from smoking at least 30 days before and 30 days after surgery - Topic therapy in skin folds (infections, intertrigo or maceration) - Discussion and signing of informed consent
2. In the 24 hours before surgery: - Complete bathroom / shower with antiseptic soap (possibly early in the morning and in any case within 24 hours) - Fasting from midnight
3. On the day: - Trichotomy 2 hours before entering the operating room - Preoperative planning - Antithrombotic therapy (low molecular weight heparin, first administration 5.000U / 30' before inducing anesthesia)
4. Intraoperative surgical strategies: - Ligation of the perforating arteries - "Quilting sutures" - Intraoperative microcirculation check with spectrophotometer laser - Compression dressing
5. Post-operative management: - Heating of the patient - Garments and compression dressing - Antithrombotic, antibiotic and pain therapy - Early mobilization of the patient - Ordinary discharge (if the weight loss is <50 Kg) - Assisted discharge (if the weight loss is >50 Kg) - Nutritional protocol - In the first 10-15 days night's sleep with suggested posture (angle of 30 degrees) and abstention from driving - Abstention from sport for at least a mounth - Cleaning shower only after the stitches' removal - Compression dressing for 4-6 weeks - It is recommended to avoid direct exposure to sunlight or heat sources for at least 4-6 weeks

Results

We reviewed a total of 78 patient charts (Table 2). There were 8 men (10%) and 70 women (90%), and the mean age was 39. 14 patients (17.95%) were smokers. 48 patients (61,54%) had a BMI >25 kg/m2 and 30 patients (38,46%) had a BMI <25 kg/m2.

With reference to the bariatric procedures used, the BMI delta was analysed between the preoperative period and 2-year follow-up, when the post-bariatric procedure was performed. The average decrease in BMI in case of gastric bypass was 40%; in case of gastric banding was 29% and in case of sleeve gastric resection was 57%. The average BMI at the time of post-bariatric surgery was 31.4 kg/m^2 in case of gastric bypass; 32.9 kg/m^2 in case of gastric bending; 28.9 kg/m^2 in case of sleeve gastric resection. So, we noticed that the average BMI at the time of post-bariatric surgery was similar, regardless of the bariatric procedure used. All these patients were asked to keep their

Table 2. Patient characteristics

Patient characteristics	N	%	Mean
Patients	78		
Males	8	10%	
Females	70	90%	
Age			39
Interval between bariatric and body contouring surgery (months)			20

weight stable for at least 6 months before body contouring surgery.

Of the 78 patients, 14 underwent more than one surgical post-bariatric procedure. We adopted the multi-staged approach that we consider more rational and prudent: the procedures were performed in more than one surgical session and the time required between two surgical sessions was 3-6 months. Surgical procedures included: abdominoplasty, medial thigh lift, brachioplasty and mastopexy. A total of 94 body contouring procedures were performed in 78 patients: 64 patients (82%) underwent 1 operation, 13 (16%) underwent 2 operations, and 1 (1.3%) patient underwent a complete body contouring (4 operations). The distribution of procedures performed can be found in Table 3. According to the criteria described above, the results were independently scored by two blinded observers. The outcomes obtained in our patients are shown in Table 4. On a total of 94 procedures performed, complications occurred in 12 cases (12,76%). There was no post-operative mortality. The surgical procedure most frequently associated with complications was abdominoplasty. Wound dehiscence and seroma have been the most common complications, which occurred in 7 and 5 patients, respectively. Among smoking patients (14 patients), complications occurred in 5 cases (3 cases of dehiscence and 2 cases of seroma), with an incidence rate of 35,71%, while in the population of non-smoking patients (64 patients), complications occurred in 7 cases (2 cases of dehiscence and 5 cases of seroma), with an incidence rate of 10,94%. In 9 cases complications occurred in patients with a pre-operative BMI >25 kg/m^2, but we have not observed a possible relationship of the complications with the bariatric technique performed.

Table 4. Classification of outcomes

OUTCOME	N	%
Excellent	31	32,98
Good	52	55,32
Moderate	9	9,57
Poor	2	2,13
Total	94	

Table 3 - Distribution of procedures performed

BODY CONTOURING PROCEDURE	TOTAL AMOUNT	MALES	FEMALES	%
Abdominoplasty	64	4	60	82,05
Abdominoplasty + Medial thigh lift	7		7	8,97
Abdominoplasty + Brachioplasty	2		2	2,56
Abdominoplasty + Mastopexy	2	2		2,56
Abdominoplasty + Belt lipectomy	2	2		2,56
Complete body contouring	1		1	1,28
Total	**78**	**8**	**70**	

Discussion

Our study showed that 25.4% of all patients had undergone body-contouring surgery after massive weight loss due to bariatric surgery. At the time of plastic surgery, the average BMI of these patients was comparable, regardless of the bariatric procedure performed, and the required weight stability time was in all cases 6 months - 1 year, so we could hypothesize that the bariatric procedure did not influence the outcome of post-bariatric body contouring surgery.

The abdominoplasty is the most required procedure, as documented in our study in which all the 78 patients submitted it. In our experience, the overall complication rate was 12.76%, with wound dehiscence (7 cases, 7.45%) and seroma (5 cases, 5.32%) being the most common complications. Among cases of seroma, it has also been reported a case of persistent lymphorrhea, in a patient with altered blood protein level and hypoalbuminemia, that has been treated with drainage followed by the use of compression garments and then with surgical revision. The operation most frequently associated with complications was abdominoplasty, and the central region of the surgical wound is particularly exposed to the risk of dehiscence. The information from the review of the international literature, regarding the post-bariatric plastic surgery, reported an overall complication rate of 44.93% (14-20), while our complication rate (12.76%) is much lower. We have noticed a deep relationship between development of complications and cigarette smoking (Table 5). In fact, smoking has detrimental effects on wound healing processes and increases the risk of wound dehiscence (21). We have observed that the incidence rate of complications among smoking patients is more than 3 times higher than among non-smokers patients (35.71% vs 10.94%).

Table 5. Distribution of complications in the populations

TYPE OF COMPLICATIONS	Smoking patients (14)	Non-smoking patients (64)
Seroma	2	5
Skin dehiscence	3	2
Total	**5 (35,71%)**	**7 (10,94%)**

Although, as previously stated, a possible relationship of the final outcomes with the different bariatric techniques performed has not been detected, we have noticed instead a connection between development of complications and pre-operative BMI. Arthurs et al. (22) found that patients with a BMI greater than 25 kg/m^2 are at nearly three times the risk of postoperative wound complications. According to this study, we selected 25 kg/m^2 as a reference BMI value. We noted that 9 cases of complications (18.75%) occurred among 48 patients with a BMI >25, whereas among patients with BMI <25 complications occurred in 3 cases (10%). We have observed that the incidence rate of complications among patients with a BMI greater that 25 kg/m2 is about 2 times higher than among patients with a smaller BMI (18,75% vs 10%). Another interesting finding is the influence of a stable weight prior to surgery. From the literature, it appears that patients having a stable weight plateau for 3 months or longer before body contouring surgery experience less complications in comparison to patients with a pre-operative variable weight (22, 23). One hypothesis is that the nutrition status is better in patients with a stable weight (24-26). These relationships with smoking, pre-operative BMI and stable weight point out that the first criterion for the prevention of complications is correlated with the selection of patients, in agreement with the literature (21-27). To select patients, we also believe appropriate to collect medical history with particular attention to the presence of comorbidity and to the use of drugs. We recommend the suspension of drugs containing acetylsalicylic acid 30 days prior to surgery and abstaining from smoking at least 30 days before and 30 days after surgery. Regardless of these variables, we would like to focus on the protocol that we have proposed for the management and treatment of patients, which requires a close collaboration between medical and nursing staff. In the pre-operative stage, it has a great importance and significance the patient's preparation as the complete bathroom / shower with antiseptic soap (possibly early in the morning and in any case within 24 hours) and trichotomy 2 hours before entering the operating room, because if the shaving is performed more than two hours before there is an increased risk of infections (28). Regarding the intraoperative surgical strategies,

we agree with the study of Rangaswamy M. (29) who suggest the routine use of "quilting sutures" to reduce the risk of seroma. In fact, this kind of multiple sutures which approximate the flap to the fascia eliminates dead space, leads to early adhesion, prevents shearing forces between the two layers and reduces the tension on wound closure. Even the ligation of the perforating arteries with clips or suture thread, instead of traditional diathermocoagulation, helps to reduce the formation of hematomas and seromas. Furthermore, we consider it necessary to place suction drains and we propose their removal after 48 hours for patients with a weight loss <50 Kg and after 5-6 days if the weight loss is greater. We recommend the use of garment or compression dressing for at least 4-6 weeks (30). In the post-operative stage, nursing staff must be properly educated about antibiotic, antithrombotic and pain therapy and about early mobilization of the patient. Finally, we cannot underestimate the nutritional aspect (30, 31). Wound healing is a nutrient-dependent process: protein plays a critical role in this process and protein deficiencies are known to lead to increased inflammation and may contribute to slow wound healing (32). But in the post-bariatric patient population there are often metabolic and nutritional deficiencies, so the use of protein supplementation is helpful to combat this problem. The literature confirms (31) that the greatest improvement in postoperative outcomes was observed in patients who received specialized nutritional support preoperatively.

Conclusion

With the increasing number of weight loss surgery, there is a higher number of patients who desire a body contouring surgery. Post-bariatric body contouring is an important component to the total care of the obese patient and it is significant to optimize results achieved from bariatric surgery. However, these patients have an important risk for complications, because of poor skin quality, comorbidities and metabolic and nutritional deficiencies. The comparison of our personal data with those of the international literature is positive and confirms the efficacy of our management protocol with regard to the prevention of complications. It is still important to point out that the main strategy in the prevention of complications is the preliminary selection of patients, based on their medical history.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Diabetes mellitus literacy in a regional community of a developed country

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Summary. *Background:* Prevalence of diabetes mellitus (DM) is on the increase. Yet discrepancies exist in research reports regarding the level of knowledge of the disease in 'rural versus metropolitan communities', and 'developed versus developing countries'. This study examines the level of general knowledge of diabetes among adult community members of a regional city of Australia, whether it is comparable to reports from low-mid income countries. *Methods:* The study was designed to be a cross-sectional day-time-population survey. Major shopping centres were chosen for convenience sampling of community's daytime population. A total of 315 participants' (154 males and 161 females) responses were received. Data were analysed using SPSS – 20 software to identify differences between sub-groups of age stratifications, educational status, gender and the participants assumed knowledgee. The participant's average knowledge of diabetes symptoms and complications were also assessed. *Results:* The major finding is that the subgroup who claimed to know 'very little' showed equivalent knowledge levels with those who thought they had 'considerable knowledge'. The females know more about diabetes management than males ($P < 0.004$); level of knowledge increased with educational status ($p < 0.01$). These observations were comparable with reports from developing countries. *Conclusions:* The limited knowledge of diabetes symptoms and complications in the population can be mitigating against early reporting of patients to diabetes clinics in the community. To ensure continuous decline in prevalence rates of diabetes and its complications, the ongoing efforts of diabetes awareness and educational programs need to be improved, particularly with regard to males and school children. (www.actabiomedica.it)

Key words: assumed knowledge, diabetes literacy, educational level, gender, rural communities

Introduction

It is noted from the Australia Bureau of Statistics (ABS) that 60% of Australians lack basic health literacy (1). Health literacy is defined as the capability to seek, understand and act on health information (2, 3). Low health literacy is commonly seen in those with low education levels and can be improved by providing necessary educational programs (3). It is known that high levels of health literacy can influence early diagnosis and prevention of diabetes (4). In particular, poor health literacy is associated with poor diabetes control as well as an inability to to seek preventive measures (5, 6), which can lead to further complications. Diabetes requires constant education and constant medical care to achieve an adequate level of glycaemic control, especially as it promotes self-efficacy and self-care behaviors and glycemic control (7).

As in 2015, Orange ranked ninth in NSW diabetes hotspot list with 2030 individuals living with diabetes and an incidence rate of 5.1% (8). According to the Socio-Economic Indexes for Areas (SEIFA), Orange is a regional area with socioeconomically disadvantaged residents (9); and the disadvantages includes

health services (10, 11). This places an emphasis on the relationship between low education and health literacy as the individuals with low SES are less likely to access treatment options within the healthcare systems (6).

Therefore, given the incidence rate of diabetes and presumed limited knowledge of diabetic complications, prevention methods involving diabetes education need to be reviewed. It is known that such preventive education can enhance early diabetes detection and improve management (3, 5, 6). There is evidence that low socioeconomic status and residence in the urban area are independently associated with an increased risk of childhood diabetes (12), but this cannot be extrapolated for diabetes knowledge in rural areas. This led our research to evaluate these factors vis-à-vis exploring the research questions:

1. What is the level of general knowledge about diabetes among individuals in the central business district of the rural community of Australia?
2. Are there differences in age, gender and education subgroupings?
3. Do the people know as much as they perceive themselves?

Method

This study was approved by the Human Research Ethics Committee of Charles Sturt University (protocol number: 400/2016/32). The study followed a cross-sectional survey design and adopted albeit restructured questionnaire from a published research (13). Further, based on the model that diabetes prevention activities in the Stockholm Diabetes Prevention Program were started with people from different walks of life (14) as well as following the methods involving study on nurses' knowledge (15, 16), convenience sampling of community members was chosen for the study. In this instance, study participants were 18 years old and above, recruited from various local shopping centres. The study excluded participants who resided out of the Orange 2800 postcode and those under the age of 18 years. Participants who were involved with the study were provided with an 11-point questionnaire, and information sheet. Implied consent was considered when the questionnaire was submitted into a secured box on site.

The questionnaire focused on four main categories of knowledge of diabetes: types, cause, management, and complications. It was composed of two Likert scale questions, five multiple choice questions, two questions required participants to identify and write the correct answer and the last two questions required participants to either agree or disagree with a statement. These questions in the survey were inspired from similar studies (17-19), to suit a general population. A total of 315 participant responses were received (154 males and 161 females).

Data were analysed using SPSS - 20 software. Besides descriptive review, multivariate analysis of variance (MANOVA) was performed to identify differences between sub-groups of age stratifications, educational status, gender and participants assumed-knowledge. In particular, responses on knowledge were allocated values for each question answered based on either the Likert scale, or number of questions that the respondents got right. These values were compared between groups. For the third research question i.e. whether the people know as much as they perceive themselves, participants were sorted and sub-grouped based on their responses to Questionnaire #2 categories and responses to questionnaire #3 were analysed to determine the percentage of each subgroup.

Results

Descriptive statistics of participants' responses by age and gender subgroupings are presented in Table 1. The participants ages ranged from 18 to 90 years old and 41.27% were 31-50 years old and had a secondary/high school education level. However, there is no difference between age-stratified groups ($p > 0.05$; Table 1a). Out of 315 individuals who took part in the study, 154 were males and 161 were females. Average score on knowledge of symptoms, complications and types of diabetes in the population were 14%, 29%, and 54%, respectively (Table 1b). On gender difference, there are more females than males who indicated more correct answers about the management of diabetes. Further, the scores on knowledge of causes,

Table 1. Descriptive statistics of age and gender subgroupings

A: Age groups categorized into educational levels

Age (years)	18-30	31-50	51-70	71-90	Total
Female/male*	33/27	66/65	49/54	13-Aug	161/154
Primary	1	5	8	4	18
High	33	64	60	15	172
Undergrad	15	23	18	0	56
Postgrad	10	28	13	2	53
Others	1	11	4	0	16
Total	60	131	103	21	315

*Further described on 'part B' of table

B: Gender differences in knowledge of diabetes mellitus

Number of correct answers			
No of answers	Male	Female	Total
0	10	7	17
1	34	17	51
2	42	46	88
3	68	91	159
Total	154	161	315
% score on knowledge of diabetes mellitus			
Knowledge base	Male	Female	Average
Causes	37.7	40.2	39
Complications	25.8	32.9	29
Management	34.6	39.6	37
Symptoms	10.5	18.1	14
Types	48.2	59.9	54

complications, management, symptoms and types of diabetes among males were lower compared to females (Table 1b: $p < 0.004$). This study therefore suggests that females in Orange have better knowledge of diabetes management in comparison to males.

A correlation between the educational level and the average correctly identified responses of symptoms and complications ($p < 0.01$) was found. Participants had to state the symptoms, which were marked according the Diabetes Australia criteria that contained the most common 13 symptoms. Nine options were given for complications from which only seven were correct consisting of long and short term diabetes complications. The other two were distractors. Those who left the question blank or circled 'don't know', scored a zero. The result shows that the higher the education level, the more symptoms and complications participants they identified (Fig. 1).

On analysis of participants' assumed-knowledge of diabetes based on the number of correctly identifiable diabetes causes, it was expected that individuals who claimed to have 'considerable knowledge' would correctly identify all itemised causes of diabetes. The observation was not the case, as no statistical significant difference was observed between groups. The participants who claim to have 'considerable knowledge' had lower diabetes knowledge when compared to those with 'some knowledge. Also, participants who claimed to have 'nothing' or 'very little' knowledge actually knew more than they thought (Fig. 2).

Discussion

This study has investigated level of knowledge of diabetes, including its complications and symptoms as well as management, among a cross-section of the Orange community represented by a convenient sample people from various shopping centres. Most of the participants were 31-50 years old, but there is no statistical difference between age-stratified groups (Table 1a). In the systematic review that evaluated the relationship of health literacy to age, educational status and gender, it was noted that some studies had observed age as a contributing variable, while some others reported no significant differences, though several reasons were adduced for these discrepancies (20). A report from low-mid income community has also indicated no significant difference between age-groups (21). Therefore, the observation from this study regarding age agrees with some published literatures. Given that health literacy screening for older adults is also recommended as valuable (22), the observation of

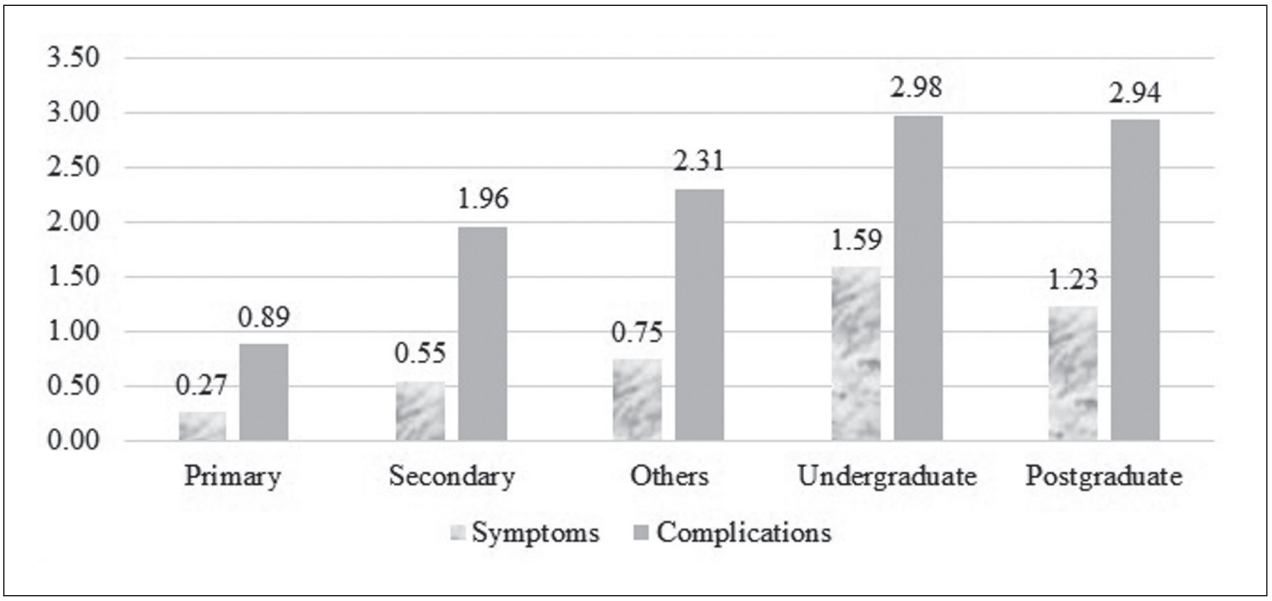

Figure 1. Educational level vs. knowledge of diabetes symptoms

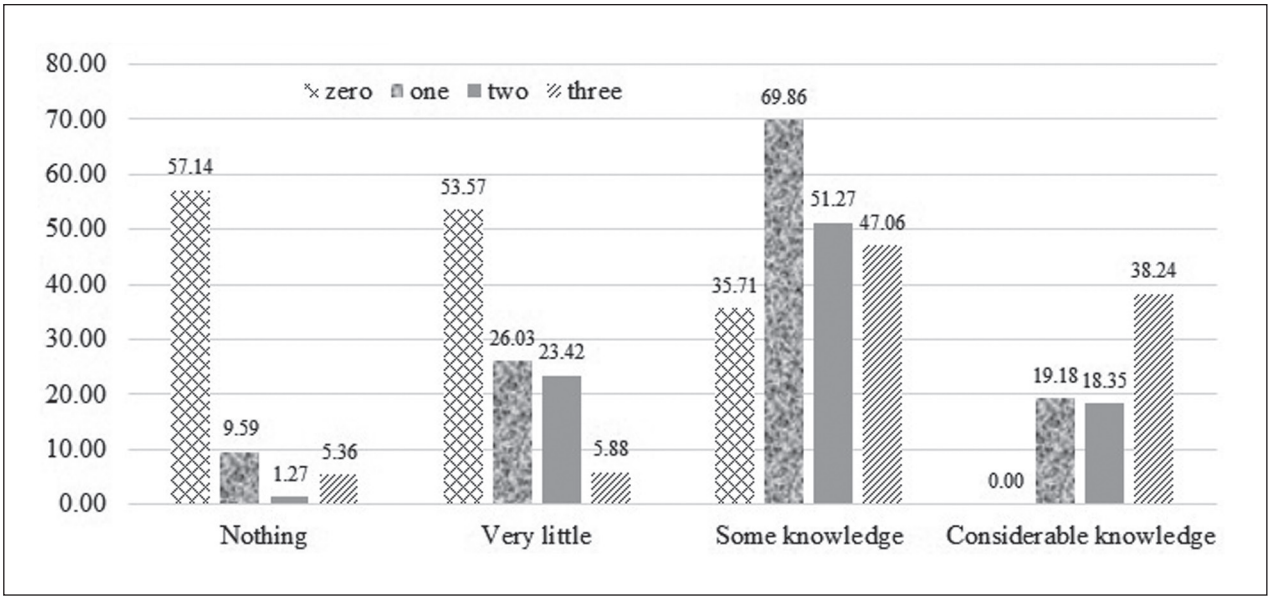

Figure 2. Assumed knowledge vs. correctness of diabetes symptoms

no statistical significant difference between age groups imply that diabetes education is equally needed across all age-groups in Orange community of NSW.

This study observed significant differences between educational status on level of knowledge of diabetes symptoms and complications (Fig. 1). This observation is in agreement with the conclusion from systematic review of several studies that educational status correlates with better health literacy including diabetes (20). Another review of literature in Australia also established that successful interventions generally consist or depends on patient education (23). Further,

a study typically similar to ours from rural communities of low-mid income countries also affirms that better education is associated with higher diabetes knowledge (24). Indeed, educational status attenuates incidence of cardiometabolic diseases including diabetes (25). Combined with the observation and inference on age, what this report adds to literature is that diabetes literacy in regional communities of developed nations may be the same as in low-mid income countries.

This study also observed significant differences between gender groups (Table 1b), that the proportion of men who know about diabetes management is less when compared to the subpopulation of women. This is in agreement with observations from another study that investigated electronic health literacy among Hispanics with diabetes, which reported level of women to be significantly higher than those of men (26). The observation in the current study is at variance with other reports. For instance, a study of low-mid income sectors indicated no significant differences between gender groups (21), while another indicated more knowledge in men than women (27). However, the report of Lemes Dos Santos and colleagues agree with our observation that women have better general knowledge of DM (28). Therefore, what this study contributes is the additional data and information that, at least, women in Orange community of Australia are more knowledgeable on diabetes relative to men.

The third and most interesting finding of this study is from evaluation of stratified groups of participants' assumed-knowledge of diabetes. The results show that individuals who claimed to have considerable knowledge of diabetes do not necessarily have better knowledge than those indicating some knowledge. This can be argued that half of the study population (51.8%) constituting the "some knowledge" cohort may have skewed the results. However, it is probably more important to note that participants who claimed to have "no' or 'very little' knowledge actually knew more than they thought. The implication is that diabetes education should be packaged with the awareness that some people know more than they claim, and vice versa. This inference is in agreement with a thesis report that even among nurses delivering diabetes care, perceived knowledge may be close to actual knowledge, but deficiency existed in terms of the required accurate current knowledge (29). The relevance of this particular study lies in the idea that perceived risk, by those who assume to know considerable much, may underestimate actual risk (30). Moreover, there may be nurses who admit to know little, but are providing diabetes education (31); as there is low level of knowledge among public health students (13). In our opinion, this affirms the recommendation for diabetes education to focus on students of all levels as well as postgraduate and practicing healthcare personnel.

Further, it is arguable that beside socioeconomic status, urban residential status is a factor in health literacy (24). In Australia, while about 33% of the populace live in rural communities, the health status of the remote and rural dwellers are generally poorer compared to those in metropolitan cities (32). The hypothesis arising from this study is that although 'health status' may different from, but dependent on 'health literacy'; health status among rural dwellers being generally poorer compared to those in metropolitan cities may be a factor of low health literacy in the rural communities.

Limitation

This study is limited by duration as it was an integrated research training for undergraduates. Hence recruitment was 'convenience sampling' limited to Orange community and daytime. However, the findings contribute to the evolving discourse that is developing to support screening for health literacy skills in clinical care, which has the potential to influence changes to clinical practice in communities (2). Hence, the relevance of this study is that health promotion programs are equally needed in the communities of both developed and low-mid income countries to increase diabetic awareness.

Conclusion

The major finding is the lack of general knowledge of diabetes complications, symptoms and management among males and females resulting in poor diabetes health literacy in the population. The limited

knowledge about diabetes symptoms and complications among the participants mitigate against early reporting of patients to diabetes clinics in the community. One of the contributions to literature is that diabetes literacy in regional communities of developed nations may be the same as in low-mid income countries. Another contribution to literature is the additional data and information that women in Orange, NSW are more knowledgeable on diabetes relative to men. A most interesting finding of this study is from evaluation of stratified groups of participants' assumed-knowledge of diabetes – whereby the results show that individuals who claimed to have considerable knowledge of diabetes do not necessarily know more than those that indicated little or no knowledge. To ensure continuous decline in prevalence rates of diabetes and its complications, the ongoing efforts of diabetes awareness and educational programs need to be improved. That is, the relevance of this study to health promotion is the provision of evidence of the fundamental need for education programs in the community to increase diabetic awareness. It is necessary for the focus of such educational need to be across age and educational strata, especially men and school to improve awareness and to reduce the prevalence of diabetes.

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Original article

Survey on smoking habits among seafarers

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Summary. *Background:* Populations living in stressful environments experience higher mortality from lung cancer and chronic diseases. Work-related stress was identified as important determinant of smoking together to health problems caused by a incorrect lifestyles. Aim of the work was to analyze tobacco smoking addiction in seafarers working on merchant ships. Only a few studies are available on this topic. *Methods:* The survey was conducted using an anonymous questionnaire. The total number of questionnaires filled-in was 1478, out of 2000 distributed (response rate 73.9 %). *Results:* About half of the sample (55.07%) has never smoked, the 28.96% is currently a smoker, while 15.97% of responders belong to the category of ex-smokers. Analysis of the number of cigarettes smoked every day based on the rank shows that captains and officers smoke more cigarettes than the crew members who smoke 10 cigarettes maximum per day. Analysis of the level of dependence among the smokers group by the Fagerström Test for Nicotine Dependence (FTND) revealed that a 89.0% of sample has a low/very low dependence level, and only the 10.98% of the sample show a high/very high dependence. A further analysis of the results of FTND in the different age groups has shown that the 41-50 and 51-60 age groups have high dependence levels. *Conclusions:* Working on board is associated to a high risk for chronic, lifestyle-related diseases, due also to lifestyle behaviours. This study suggests to further investigate the presence of other risk factors such as diet, physical exercise, combined with tobacco smoking, obesity. (www.actabiomedica.it)

Key words: seafarers, smoking habits, nicotine addiction, lifestyles, prevention, public health

Introduction

World Health Organization (WHO) has estimated that tobacco use, smoking and smokeless is currently responsible for the death of about six million people across the world each year with many of these deaths occurring prematurely (1-6), and tobacco-related diseases even claim more lives than AIDS, malaria and tuberculosis combined (7).

Active smoking and involuntary smoking have been reported to be associated with the development of many forms of cancer, cardiovascular diseases, chronic obstructive pulmonary disease (COPD), complications of pregnancy, sudden infant death syndrome, etc (8-10).

Populations living in stressful environments, smoke more heavily and experience higher mortality from lung cancer and chronic obstructive pulmonary disorder. Stress in general and work-related stress in particular were identified as important determinants of smoking (11, 12) together to health problems caused by a lifestyle based on incorrect knowledge towards food, drugs, alcohol (13-16).

Research on the relationship between working

conditions and smoking has demonstrated that smoking and occupation are related each other and that work–family conflict may be associated with increased levels of smoking (17-19). Other studies have found significant relationship between working time and smoking (20-23).

Seafarers are often represented in popular literature as heavy smokers and drinkers. Fishermen and merchant seamen are markedly subject to alcohol and cigarette consumption.

Addiction is common among seafarers as well as increased risk of occupational accident in this population (24). Many factors favor the use of drugs in seafarers: geographical remoteness, social isolation, stay on the ship, dangerous situations, stress, sleep disturbance, etc. The search for positive effects such as the stimulant effect in the fight against fatigue, or negative, as in escape from stressful situations is therefore important for seafarers. Stressful situations affect often seafarers living in an isolated and hostile environment, which involves high risk and requires vigilance and rapid reactions. For example, the use of alcohol or drugs represents a risk factor for occupational accidents, particularly fatalities, and premature deaths (25-29). For fishermen, mean voyage time has been significantly associated with nicotine dependence. The number of days spent at sea during the past 12 months has been significantly associated with nicotine dependence (25).

Health problems of seafarers can be caused both by their occupational exposure (e.g. kind and frequency of contact with hazardous substances, stress level onboard) and by lifestyle-related behavior (smoking, alcohol) (30) or incorrect eating habits (31-34). Even the misuse of drugs can sometimes cause serious allergic effects (35-40). The seafarer's cigarette consumption is considered as an important CVD risk factor higher than in the general population (41).

Besides accidents and work-related injuries, severe CVD represent a main cause of death, both in general population from industrialized countries as well as in seafaring population (42). These findings were also confirmed by the investigation on the main causes of death on board ships assisted by Centro Internazionale Radio Medico (C.I.R.M.), the Italian Telemedical Maritime Assistance Service (TMAS) (24). Working at sea is associated with specific psychosocial stressors (including long-time separation from the family, mental stress, extremely long working hours, permanent changing crews, reduced duration and quality of sleep on board, irregular working time, and permanent physical impacts such as ship movements, noise and vibration) (42). The additional effect of adverse lifestyle factors such as smoking, sedentary lifestyle, and obesity, which is also prevalent among seafarers, is regarded as multiplicative factor (43-45).

This paper has analyzed the problem of tobacco smoking addiction in seafarers working on merchant ships. Only a few studies are available on this topic, dealing with this issue and they refer to French, Spanish, Greek, Italian, Polish, Scottish and Turkish seamen (46-52). Analysis was done on a sample of seafarers working for three Italian (Finaval, D'Amico and Carboflotta), and one French (CMA CGM) shipping companies, investigating not only the tobacco smoking addiction among seafarers but also their level of knowledge and awareness of the risks for health.

Materials and methods

Study population and data collection: The survey was conducted among seafarers of the shipping companies above described, using an anonymous questionnaire. The questionnaire was sent to 80 vessels (with an average of 25 seafarers on board, for a possible sample of 2,000 people as a sample for the interview). Each seafarer received a sealed envelope containing a letter explaining the purpose of the survey, the questionnaire and instructions on how to fill in it (53). The questionnaire validity was tested by administering it to seafarers of different rank to evaluate the "face validity". The questionnaire was distributed after health education interventions (seminars) designed to make seafarers aware of health risks linked to incorrect lifestyles, with the aim to improve the working conditions quality, and occupational safety on board ships.

The questionnaire was completely anonymous and filled only on voluntary basis. After completing the questionnaire, each participant was requested to return it in its original envelope and seal without marking. All closed envelopes were gathered by the captains and

then sent to the Epidemiology group of University of Camerino (UNICAM) for data analysis. The study was conducted according to the Helsinki Declaration.

Survey tools: The questionnaire included three parts. The first one was centered on seafarer's personal data (sex, age, nationality, education and rank), and asked questions about the awareness of injuries and diseases caused by tobacco smoking, habits and lifestyle. The second part was for current smokers only and included specific questions about tobacco consumption (e.g. at what age did you begin smoking? How many cigarettes do you smoke? Do you currently want to quit smoking?), and tobacco addiction. In this section, the degree of nicotine dependence in current smokers was also assessed by the Fagerström Test for Nicotine Dependence (FTND) (54). The Fagerström Test is composed of six items. Questions requiring an answer yes/no are scored from 0 to 1, whereas multiple-choice items are scored from 0 to 3. The items are summed to yield a total score of 0-10. The global Fagerström score assesses the intensity of physical nicotine addiction: low dependence (0 to 2 scores), medium dependence (3 to 4 scores), high dependence (5 to 6 scores) and very high dependence (7 to 10 scores). A score of ≥ 6 was considered as a cut-off to asses a high nicotine dependence (55). The last part of the questionnaire examined ex smokers and how they manage to quit smoking (in this paper, only current smokers will be examined).

Before the distribution, the questionnaire was validated by a previous pilot study performed on 12 seafarers working onboard cargo ships. This to assess its face validity (56).

Analysis: Questionnaire answers were transferred into Microsoft Excel sheets. This software was used for data storing and processing. Statistical analysis was performed by the X-L stat software (57). Descriptive statistics were used to analyze the distribution of variables. Qualitative data were described using frequencies and percentages. The Chi-square analysis and the Odds Ratio, to evaluate the differences between ages, rank, educational level, alcohol use and tobacco dependence level, has been applied. The level of statistical significance was set at $p<0.05$.

Results

The total number of questionnaires filled-in was 1478, out of 2000 distributed (response rate 73.9%).

Table 1 shows the socio-demographic characteristics of the sample.

About half of the sample (55.07%) has never smoked, the 28.96% is currently a smoker, while 15.97% of responders belong to the category of ex-smokers. A great part of the sample (92.8 %) is aware that smoking cigarettes, or other tobacco products, seriously compromises health. Among the main risks associated with tobacco smoking, lung cancer was indicated by the 92.02% of participants, followed by cancers of the mouth, larynx, and esophagus (53.45%), hypertension and heart problems (52.44%), depend-

Table 1. Demographic characteristics of interviewed workers

Gender	n.	%
Male	1478	100.00
Female	-	-
Age		
<=20	87	5.89
21-30	443	29.97
31-40	423	28.62
41-50	330	22.33
51-60	184	12.45
>=61	11	0.74
Nationality		
Italian	182	12.31
Indian	679	45.94
Filipino	486	32.88
English	5	0.34
East Europe	93	6.29
Chinese	14	0.95
French	19	1.29
Educational level		
Elementary	54	3.65
Middle school	198	13.40
High school	425	28.76
Professional diploma	189	12.79
Occupational training	581	39.31
Degree	31	2.10
Rank		
Captain	70	4.74
Deck officer	251	16.98
Engine room officer	87	5.89
Deck crew	550	37.21
Engine room crew	408	27.60
Galley and catering crew	112	7.58

ence (42.96%), increase in cholesterol and risk of cardiovascular disease (22.19%), nervousness, apathy, anxiety and headache (16.31%). Among the diseases less associated with tobacco smoke by interviewed people, there were mucous hyper-secretion (catarrh) (10.83%) and conjunctivitis (7.24%). The 1.83% of the sample answered 'do not know'.

Concerning health problems declared by interviewed people, 4.53% of responders reported suffering from coughing fits (32.84% of them, productive). Among seafarers asserting to have dyspnea (35 people, representing 2.37% of the sample), 25.71% of them declared dyspnea at rest, and 42.86% of them after an effort. The 6.97% of responders declared to expectorate in the morning. The 88.70% of those who completed the questionnaire stated that cigarette smoking addiction is caused by nicotine, the 23.68% thought that addiction is also due to tar and carbon monoxide, while the 4.74% of the sample did not know at all what ingredient is involved in addiction.

Examining the sample by age, the majority of current smokers belongs to 21-30 years old group (33.88%), while former smokers belong to 31-40 years (31.36%). Among current smokers, the majority of smokers are deck crew members (35.28%), followed by engine room crew (26.40%), and deck officers (19.86%).

Table 2 shows the correlation of smoking with age, educational level, rank, and alcohol use. The prevalence of smokers was significantly related with the educational level, the working rank and alcohol use. No significant values were noticeable for the age variable. The higher Odds ratio was obtained comparing the alcohol use and smoking.

In terms of number of cigarettes smoked every day, 11.69% of people declared to smoke from 21 to more than 30 cigarettes per day. The majority of interviewed people smokes 1-10 cigarettes (63.79%), followed by those who smoke 11-20 (24.53%). Analysis of the number of cigarettes smoked every day based on the rank shows that captains and officers smoke every day more cigarettes than the crewmembers who smoke 10 cigarettes maximum per day.

Comparing the number of cigarettes smoked per day with the age of subjects working in the captain category or in the crew category, no statistically significant correlation between the number of cigarettes smoked and the age of the subjects was observed. In the crew category, a statistically significant correlation between age and number of cigarettes smoked per day has been found (Pearson value = -1.000; p=0.0001). This correlation is negative, in particular when comparing the age and the number of cigarettes in the range 1-10 cigarettes per day.

The analysis of the results of FTND in the different age groups has shown that the 41-50 and 51-60 age groups have high dependence levels (18.09% and 19.61% respectively). Considering the rank, the cap-

Table 2. Correlation of smoking with the demographic factors

	Number (percent)				
Variables	**Smoker**	**Non smoker**	**P-value**	**OR**	**95%CI**
Age					
Less than 30	171	300	0.285	1.14	0.899 to 1.380
More than 30	257	514	0.285	0.88	0.639 to 1.120
Educational level					
Elementary/middle school	74	155	0.0002	**1.76***	1.379 to 1.981
Equal to High school	354	1305	0.0002	0.57	0.269 to 0.871
Rank					
captain/officer	135	189	0.0019	**1.52***	1.259 to 1.781
crew	293	625	0.0015	0.66	0.399 to 0.920
Alcohol					
No drink	22	91	0.0042	0.47	0 to 0.995
Outside of meals, drunk, often	98	190	0.0042	**2.13***	1.604 to 2.656

* Correlation significant of the P (α<0.05)

Table 3. Odds Ratio of single ranks correlating with the medium/high FTND value

Rank	P	OR	95%CI
Galley and catering crew	**0.0225**	0.50	1.430 to 2.549
Engine room crew	**0.0056**	0.43	1.421 to 2.559
Deck crew	**0.0001**	0.31	1.409 to 2.571
Captain	**0.0002**	**2.45***	1.523 to 2.457

*Correlation significant of the P (α<0.05)

tains and the deck officers are the groups characterized by high dependence (30.00% and 22.35% respectively).

Comparing the results of FTND in the captains with those obtained in the deck crew, engine room crew, and galley/catering crew, the captains showed a risk of developing a medium/high dependence two times higher than other ranks (Table 3).

Subjects, included in the smoker group, were specifically interviewed also about the awareness of smoking. The 33.41% of seafarers declared to smoke little, the 40.89% enough, the 19.63% a lot, and the 6.1% very much. Cross-checking the dependence and the awareness of it, the highest percentage (44.68%) for the "enough" level of awareness revealed a high/very high dependence. The lowest percentage (6.38%) for the low level related with high, and very high dependence has been recorded.

In terms of job position, the awareness of smoking was statistically analyzed in comparison with the single ranks, processing the data with the Chi square Test. In the galley crew an Odds ratio, relatively to the unconsciousness of smoke dependence, five times higher (Odds ratio = 5.92, p=0.0002) than Odds ratio obtained in the captain rank (OR= 0.17, p=0.0002) was noticeable.

In terms of desire to stop smoking, the 68.09% of seafarers classified in the high/very high dependence level answered that they want to quit smoking. Among this group, the 68.7% has tried to quit, and 95.4% of them restarted to smoke thereafter.

Discussion

This study showed that a low percentage (about one fourth) of seafarers interviewed on board of merchant ships were smokers. This percentage was lower than the general prevalence rate of smoking among seafarers (51, 52). Data processing showed a good perception about the possible effects of tobacco smoking on health. In particular, this awareness concerns cancer (lung, mouth, larynx, and esophagus), cardiovascular diseases (hypertension, heart problems, high cholesterol, etc), nervousness, and anxiety.

The relationship between the prevalence of unhealthy lifestyles, as smoking with some pathologies and ranks on board was further evaluated. This as some studies highlighted a positive correlation between the onset of pathologies linked with an elevated blood cholesterol, high blood pressure, fat in food, smoking, and the different job positions (58, 59), whereas other studies reported only a marginal relationship (60).

In terms of rank, merchant ships should be considered as a working environment to be kept under control because stressful, and characterized by a high prevalence of smokers than general population ashore (51, 61). Even if the prevalence of smokers between the seafarers employed on board of the merchant ships, enrolled in this epidemiological study, is not very high, the smoking habits seem to be circumscribed in specific categories of our sample. The highest percentage of current smokers on board is in the range of age between 21 to 30 years. Other studies have reported a statistically significant correlation between smoking and educational level (52, 62-65), whereas a similar correlation was not found in relation to the different nationality of subjects. In terms of activity on board of merchant ship our data indicate that the seafarers employed as crew were the smokers more at risk. Analyzing the data on the number of cigarettes smoked per day, the captain and officers were the strongest consumers, smoking the maximum number of cigarettes (21 to 30 cigarettes per day).

In contrast, crew members heaviest smokers took 10 cigarettes per day. The worrying result was the high prevalence of hard smokers among captains and officers. These results were confirmed by statistical analysis because people with a role of control and high responsibility showed a positive statistically significant correlation with the smoking habit, with an increasing Odds ratio compared with people with a lower rank.

Furthermore, the crew people were those with the higher number of subjects which never smoked or stopped smoking. These data can be explained considering that captains and officers have more freedom to

choose when and how much to smoke. Another worrying result is the positive and statistically significant correlation between smoking and alcohol use. Probably, the contemporary presence of the smoking and alcohol habits indicates that the subjects which have the necessity to smoke perceive also the necessity to drink alcohol to tolerate the stress or fatigue. These findings are consistent with those of other studies suggesting a possible correlation between the increase of smoking and alcohol use, and the work-related stress. Alcohol and cigarettes could also be used as anti-anxiety or antidepressant agents to relieve the impact of job stress, and to self-medicate physiological effects induced by stress (elevated cortisol, suppressed serotonin, and catecholamine secretion) to achieve the internal homeostasis (65, 66).

In terms of age analysis, younger crews represented the main smokers. In fact, a positive statistically significant correlation was found between the younger age groups (<30 years) and smoking. These findings could be explained because even if the number of cigarettes smoked by younger crews is not elevated, the frequency of smoking in this category is higher than frequency registered in the other crews (>30 years). The observation that younger people are the main smokers is worrying as they are at risk of dependence because of their low awareness of how much they smoke.

A less straightforward question is whether seafarers are also at heightened risk of dependence if employed in the higher ranks. Besides to the raw analysis on the number and the frequency of cigarettes smoked for single ranks and single range of age, the level of dependence has been analyzed.

The FTND showed that all people interviewed has been assessed on a low or very low dependence level.

As regards as the awareness, the galley crew has been associated with six times less awareness than captains on the amount of smoking. Compared to the engine room crews, a statistically significant correlation between this category and the captains, was not found.

Although this study has some limitations, such as the choice of sample (convenience and non-randomized) that could represent a possible bias, our results are in agreement with data of literature, in particular concerning the correlation between smoking and alcohol use, strengthening the relationship between the high levels of nicotine and alcohol consumption in seagoing workers (30, 51, 67). Probably, the free availability of cigarettes on board, could be another element contributing to continue or to start smoking between seafarers (25, 68, 69). Sailors work away from home for a long time and they do not benefit from many anti-smoking policies or rules that are the most smoking cessation interventions for the general population (63).

Traditionally, the seafaring has been perceived by the public as a risky activity, mainly because of the shipping accidents and recently the piracy. Only in recent studies, the work on board of ships has been associated to a high risk for chronic, lifestyle-related diseases, such as cancer and coronary heart disease, due to a high exposure to chemicals as well as sunlight, but also to lifestyle behaviours, such as smoking, alcohol consumption and diet (25, 30, 51, 70). Therefore, the results of this study suggest to further investigate the presence of other risk factors such as diet, physical exercise, combined with tobacco smoking, and obesity (71).

All these considerations highlight the strong need to activate prevention campaigns directed towards this particular working category. A first approach is represented by the 'Healthy ship' project (Health Protection and Safety on Board Ships), an initiative of CIRM (Centro Internazionale Radio Medico, the Italian Telemedical Maritime Assistance Service) and the University of Camerino, focused on the prevention of diseases on board ships through information campaigns about the major health risks for seafarers and their prevention (13, 14, 72, 73)

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

High sensitivity versus low level of vancomycin needs to be concern for another alternative anti- *Staphylococcus aureus* as the first- line antibiotic

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Summary. *Background and aim:* Vancomycin has been the first-line therapy for MRSA infection disease for many years. According to standard guidelines, the therapeutic vancomycin trough concentration should be above 10 mg/L and optimally between 15-20 mg/L. The aim of this study was to evaluate vancomycin trough level concentration in patients infected with MRSA. *Methods:* This cross- sectional study included a sample of 170 patients admitted to the ICU of Loghman hospital. We used a standard questionnaire, then applied appropriate statistical tests. All collected data had been analyzed and interpreted by IBM SPSS Statistics 19.0. *Results:* Among this study population, 71.8% was male. Just 20.8% of the patients can reach the therapeutic level trough even after changing the dose. It should be noted that a significant percentage of toxicity was observed after increasing the dose. *Conclusions:* Even though high sensitivity against vancomycin disc has been seen in antibiogram tests, sufficient efficiency has not been distinguished, in the sense that, just a few patients by low trough level concentration, reached to therapeutic level after the dose change. Based on some sources, because of the side effects and limited safe range of vancomycin, we should consider a new approach to the alternative antibiotics. (www.actabiomedica.it)

Key words: vancomycin, trough level, MRSA, toxicological intensive care unit

Introduction

Vancomycin is a glycopeptide antibiotic which has been used since the 1950s against gram-positive bacteria especially MRSA (methicillin-resistant *Staphylococcus aureus*) (1, 2). MRSA can cause different infectious diseases such as sepsis, endocarditis, osteomyelitis, infection of tissues, skin, and different kinds of pneumonia including aspiration pneumonia and Ventilator associated pneumonia (VAP) (3, 4). Nowadays, vancomycin is the first-line therapy and gold-standard treatment for MRSA infection disease (5-7). Vancomycin level trough has a limited range. In order to achieve appropriate and safe clinical response, IDSA (Infectious Diseases Society of America)/ASHP (American Society of Health System Pharmacists') have published guidelines in 2009(8). According to these, the therapeutic vancomycin trough concentration should be above 10 mg/L and optimally between 15-20 mg/L. Furthermore, vancomycin associated nephrotoxicity usually happens when the measured concentration of the drug is higher than 20 mg/L (9-11). The relationship between trough level and efficacy of vancomycin, or microorganism eradication is not supported by clin-

ical data, but for adverse effects prevention, trough level monitoring is necessary. There is a especial concern about the adverse effects of vancomycin treatment for infected patients. The red man syndrome and nephrotoxicity are the most important problems, which can be caused by this broad spectrum antibiotic (12, 13). While vancomycin associated nephrotoxicity is usually reversible, it may increase the medical costs, the average length of stay in hospitals, and in rare cases lead to dialysis treatment and finally death. Vancomycin dose, duration of treatment, and personality characteristics of patients are the most important factors for nephrotoxicity (14-16). For these reasons, vancomycin is one of the most-studied antibiotics in the world (17, 18). The prediction and prevention of drug toxicity according to serum level concentration is the controversial issue. This process is time-consuming and costly because of sample collection and data analysis, write order, and interpretation of results. Dramatically, due to last year`s reports in Loghman hospital, the most evaluated vancomycin trough level has shown a range between 4.5-7 mg/L, which is not acceptable according to scientific guidelines. The aim of this study was to evaluate vancomycin trough level concentration in patients infected with MRSA.

Methods

This cross-sectional study was approved by the ethical committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (Research project number 47.2018.3.11). The study included a sample of 70 patients admitted in the ICU department of Loghman hospital as well as 100 medical records of patients who were admitted and discharged during 3 years from Jun 2015 to March 2017. All poisoned patients with infection who were referred to the ICU were included in the study. Nephrotoxic patients and those who had cancer as well as patients who used another antibiotic instead of vancomycin were excluded. Among all the patients, 122 (71.8%) were male. We used a standard questionnaire including clinical, demographic data and Lab tests sections. Patient data including age, gender, vancomycin doses, hospital length of stay, vancomycin treatment length, the type of toxicity, vancomycin trough level concentration, serum creatinine, and culture results (blood, tracheal, and urine culture) were collected from their medical records. The type of microorganisms causing infection were classified as *staphylococcus*, *streptococcus*, *gram-negative bacilli*, and fungus. In addition, the antibiogram test results were recorded for 121 infected patients, and the sensitivity against vancomycin was detected. Aspiration pneumonia, ventilator-associated pneumonia, and sepsis as *staphylococcus aureus* associated infections were considered. In order to evaluate vancomycin effectiveness, the vital signs consisting of body temperature; pulse rate, respiratory rate, Glasgow Coma Scale (GCS), blood pressure; and laboratory tests including urea, creatinine, WBC, Na, K, creatinine phosphokinase (CPK) were recorded the day before treatment and 3 days after treatment. Rhabdomyolysis was defined as the CPK above 1000 U/L. The type of toxicity, which is classified as opiate overdose, drug stimulant toxicity, benzodiazepines toxicity, and mixed drug toxicity, were precisely documented. ECL (electrochemiluminescence) measured evaluable trough concentrations. If the trough levels were under therapeutic range, we measured these for a second time after dose changing. Although the AUC/MIC ratio is the best predictor of vancomycin efficacy, we could not afford it financially. Appropriate statistical tests such as paired T-test, chi-square, Wilcoxon signed-rank test, and exact Fisher tests were applied. All collected data were analyzed and interpreted by IBM SPSS Statistics 19.0.

Results

Among this study population, 71.8% was male. The mean hospital duration was 19.12 days (median=14.5). The most common poisoning was opioid overdose (30%). The next important agents have been shown in Table 1. Only 13 patients had an underlying disease such as depression, hypothyroidism, and brain tumor. Defining leukocytosis as WBC count ≥10000, pre- treatment 51.8% had leukocytosis, while this figure dropped to 41.8%, 3 days after treatment (p value=0.03). In the other hand, leukopenia which indicates severe infection, was observed in just five patients at first and then in one person after 3 days (Table 3).

Pulmonary involvement was detected in 96.5% of the study population in the forms of aspiration pneumonia (AP) (53.5%), VAP (74.1%); some of them were both dependent on the onset. In 29.7% of patients, rhabdomyolysis had occurred. The mean body temperature on the day before treatment was 38.02 c (36c - 40.3c), and 37.6^{0}c at 3 days after treatment (36c - 40c), which indicates a significant change (p value=0.0001). According to some standard definitions, when the second measurement shows the figure 0.3 times rather than the first measurement, creatinine rising was occurring. According to this, 48 people (28.2%) had creatinine rising. Serum creatinine levels were measured 3 times. The initial average was 1.18 (0.5 -9.5), subsequent average was 1.24 (0.5-11.3) and the third, post-dose change average was1.23 Showing no significant differences between creatinine levels. Generally, 96 (59.4%) patients were *Staphylococcus aureus* positive in tracheal, urine and blood culture. Among 170 patients, approximately 90% had positive tracheal culture consist of 45.3% *Staphylococcus aureus*, 34.1% *gram- negative bacilli*, 10% *streptococcus*, 1.2% fungus and 9.4% had negative culture. From 18.9% people with positive blood culture, 11.2% had *Staphylococcus aureus*, 7.1% had gram-negative *bacilli*, 0.6% had *streptococcus*. Urine culture was positive in 26.5% patients. This figure is consisted of 21.2% *gram- negative bacilli*, 2.9% *Staphylococcus aureus*, 1.2% *streptococcus* and 1.2% fungus. The most common dose of vancomycin prescribed was 1 gr.bd (81.7%). Totally, 48 patients with low trough level concentration were required to change the dose of vancomycin. The average duration of drug consumption was 6.3 days (3-90 days, SD=3.3). The most commonly measured first trough level concentration was 13.8 (0.01-55.4, SD=9); concentration, for the second measurement was 16.3 (SD=9) (Table 2). The antibiogram test was performed for 91 cases that had positive tracheal culture. These results showed 96.8% sensitivity and 3.2% resistance against vancomycin disc. In the case of urine culture, these percentages were reported to be 71.4% sensitive, 14.3% intermediate and 14.3% resistant. The sensitivity reporting for

Table 1. Patient characteristics

Gender	Number/percent
Male	122 (71.8%)
Female	48 (28.2%)
Kind of drug toxicity	Opioids 51 (30%)
	BZD 30 (17.6%)
	Stimulants drugs 7 (4.1%)
	Opioids and BZD 37 (21.8%)
	Other agents** 25 (14.7%)
	MDT * 8 (4.7%)
	Opioids and stimulants drugs 12 (7.1%)
Average hospital duration	19.12
Vancomycin sensitivity (Tracheal Culture) (Disc diffusion method)	Sensitive 91 (96.8%)
	Resistant 3 (3.2%)
Vancomycin sensitivity (Blood Culture)	Sensitive 20 (100%)
Vancomycin sensitivity (Urine Culture)	Sensitive 5 (71.4%)
	Intermediate 1 (14.3%)
	Resistant 1 (14.3%)

* Multi drug toxicity such as the mixture of sodium valproate, acetaminophen, Benzodiazepines, …
** Others agent included: Aluminium phosphide, Tricyclic antidepressants (TCAs), Carbon monoxide (CO), Acetaminophen

Table 2. Patient's vancomycin serum level concentration

Trough level concentration	<10 mg/l	10-15mg/l	15-20 mg/l	>20 mg/l	Total
First measurement	70 (41.17%)	45 (26.4%)	27 (15.8%)	28 (16.4%)	170
Second measurement	12 (25%)	12 (25%)	10 (20.8%)	14 (29.2%)	48

Table 3. Comparison of Leukocytosis, Leukopenia and fever, before and after the treatment

	Before treatment	3 days after treatment	p value
Leukocytosis	88 (51.8%)	71 (41.8%)	0.03
Leukopenia	5 (2.9%)	1 (0.5%)	
Fever	138 (81.2%)	100 (58.8%)	0.0001

blood culture was 100%. The relationship between sex ratio and trough level concentration is not significant (p value=0.2). Based on body temperature ≥37.5c defined as fever, before and after treatment, 81.2% and 58.8% of cases had fever, respectively (p value=0.0001).

Discussion

As the incidence of MRSA infection rises, the prescription of vancomycin as the first line therapy has increased. Vancomycin still is one of the most studied antibiotics in the world because of its limited safe range and side effects (5-7, 19-22). According to published data, there is a strong relationship between high doses vancomycin therapy and nephrotoxicity. However, studies have suggested that nephrotoxicity usually occurred at vancomycin levels above 20 mg/dl, thus the target dose suggesting by standard guidelines 15-20 mg/dl may increase the risk of nephrotoxicity. Additional factors may include co-ingestion of nephrotoxic agents, duration of therapy, and underlying physiological impairment (15, 19, 23, 24). We did not detect the expected any significant differences in the serum creatinine levels (p value=0.4). We realized that rhabdomyolysis may cause initial creatinine rise. The specific conditions of our patients may justify this position, most poisoned with nephrotoxic substances as confounding factors. Although it has been claimed that vancomycin-associated nephrotoxicity is usually reversible, our previous studies showed in some cases with rhabdomyolysis, the nephrotoxicity was irreversible (25). Ultimately, this is a controversial issue because of the differing definitions of nephrotoxicity. Some studies such as Lodise et al. have indicated the association between other drugs such as aminoglycosides and amphotericin B with nephrotoxicity (26). In our study, we tried to exclude the patients who used other antibiotics or nephrotoxic drugs.

According to Talaie et al. a significant number of patients in the poison center, who were infected by MRSA, could not reach an appropriate trough level after vancomycin therapy. Their results have shown the most of the patients had a trough level between 4.5-7 mg/l, which is not an acceptable level. This pilot study prompted them to carry out additional research and design this study (25).

The prevalence of *staphylococcus aureus* is 59.4% of all microorganisms in blood, tracheal and urine culture. This is the most common germ in our study. Even though in antibiogram test, the high sensitivity against vancomycin disc has been seen, sufficient efficiency has not been distinguished. In the sense that, just a few patients with initial low trough level, reached the therapeutic level after the dose change. As a study shown that the length of hospital stay (LOS) had a significant effect on vancomycin side effects, we should mention that LOS was an appropriate indicator of morbidity and mortality(27).

Eun Young Choi's study found that in patients infected with MRSA nosocomial pneumonia, the rate of relapse was high and the clinical outcome was not effective enough when treated with vancomycin (28). Some suggest that given the high cost and long term consumption required by vancomycin, as well as its side effects and limited safe range, we should consider a new approach to the alternative antibiotics (29, 30). In addition, the use of an alternative medicine to vancomycin, may not require such monitoring (9).As in recent years , the dominant microorganisms of the toxicological ICU, have been and still are *staphylococcus aureus*, the need for an effective antibiotic as an alternative to vancomycin is felt.

The most important issue to be noted in the present study is that, after changing dose in order to achieve optimum outcomes, not only did the toxic level occur, but also in some cases, the therapeutic level was not obtained. It is necessary to mention that some complications such as red man syndrome, leukopenia and pancytopenia, as vancomycin side effects, were not reported in this study.

We had several limitations in this present study; first, the study population was relatively small. It would be better to enroll more people in order to increase the study power. Second, we lost some of the cases during the study because of high turnover in our ward or expiry. Third, some of the patients have other multidrug-resistant pathogens as a confounding factor, for example, carbapenem-resistant Acinetobacter and extended-spectrum beta lactamase-positive Gram-negative organisms, although these were treated well. And finally one of the limitations of our study was that the E-test was not available for a long time due to economic sanctions.

Finally, the logical conclusion from this study is that alternative anti- staph broad-spectrum antibiotics are immediately necessary.

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Update

Umbilical reconstruction: different techniques, a single aim

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Summary. The umbilicus is a unique physiologic scar of human life resulting from the healing process of the cut umbilical cord at birth. Its absence leads to an unnatural abdominal appearance, and an abnormally shaped or misplaced umbilicus may draw undue attention to the central abdomen. Loss of the umbilicus can be an embarrassing deformity; this occurs when older techniques of umbilical hernia or incisional hernia repair are employed and after abdominoplasty, urachal cyst repair, omphalocele repair, gastroschisis repair, some tumor excisions, and mobilization of bipedicled or bilateral TRAM/DIEP flaps for breast reconstruction. Umbilicoplasty, in which the umbilicus remains anchored to the deep abdominal fascia but is transposed through a newly-formed aperture in the upper abdominal skin flap, is performed in abdominoplasty either for abdominal flap harvest or purely for aesthetics. On the other hand, umbiliconeoplasty describes the de novo creation of an umbilicus that is absent for either congenital or acquired reasons. The optimal umbilical reconstruction should be reliable, reproducible, aesthetically appropriate, and associated with low morbidity. Ideally, it is also single-staged, except in the case of an infected wound, in which case a delayed primary approach may be prudent. (www.actabiomedica.it)

Key words: umbicoplasty, umbiliconeoplasty, plastic surgery

Introduction

The umbilicus is a unique physiologic scar of human life resulting from the healing process of the cut umbilical cord at birth. It is, therefore, the first scarring to occur over the course of a human lifetime. The umbilicus is usually a round to oval depressed structure with an average diameter of 1.5 to 2 cm (1) that greatly contributes to the aesthetic appearance of the abdominal wall. It also serves as a natural reference point, lying anatomically at the crossing of the midline and the line transecting the superior iliac crests (or just superior to this line) (2, 3).

Formation of the umbilicus results from the cancellation and retraction of the following four elements: the left umbilical vein, which heads superiorly towards the round ligament of the liver; the obliterated urachus, which directs inferiorly; and the two umbilical arteries, each of which runs laterally to the corresponding internal iliac artery (4, 5). The umbilicus defines the median abdominal sulcus and contributes to the curved shape of the inferior abdomen. Its absence leads to an unnatural abdominal appearance, and an abnormally shaped or misplaced umbilicus may draw undue attention to the central abdomen (6). Loss of the umbilicus can be an embarrassing deformity; this occurs when older techniques of umbilical hernia or incisional hernia repair are employed and after abdominoplasty, urachal cyst repair, omphalocele repair, gastroschisis repair, some tumor

excisions, and mobilization of bipedicled or bilateral TRAM/DIEP flaps for breast reconstruction (7-11).

Principles of reconstruction

Umbilicoplasty, in which the umbilicus remains anchored to the deep abdominal fascia but is transposed through a newly-formed aperture in the upper abdominal skin flap, is performed in abdominoplasty either for abdominal flap harvest or purely for aesthetics. Most surgeons use a circumumbilical incision to preserve the fixed umbilicus, passing it through a new orifice in the upper abdominal flap (2). Reinsertion of the umbilicus into the abdominal wall is performed through a circular, oval, vertical (12) or transverse (13) incision. On the other hand, umbiliconeoplasty describes the de novo creation of an umbilicus that is absent for either congenital or acquired reasons (14). The optimal umbilical reconstruction should be reliable, reproducible, aesthetically appropriate, and associated with low morbidity. Ideally, it is also single-staged, except in the case of an infected wound, in which case a delayed primary approach may be prudent. Umbiliconeoplasty may be performed using any number of techniques and may include the use of flaps or grafts or healing by secondary intention (15).

Surgical techniques

Umbilicoplasty

Abdominoplasty is a common body contouring procedure with numerous reported options for umbilical management. The earliest reports in the literature described excision of the umbilicus en masse with the abdominal pannus, with or without its replacement as a graft (14, 16). Moreover, even recent reports describe umbilical sacrifice as part of abdominal pannectomy, usually in the case of a particularly large pannus and elongated umbilicus that is unlikely to survive transposition due to vascular insufficiency (17). Others describe de-epithelialization of the umbilicus with dermabrasion, followed by the creation of a scar-free umbilical depression through the securing of a circular, defatted area of the abdominoplasty flap to the umbilical base (18).

Umbiliconeoplasty with external scars

The scars are placed in variable directions. Such scars, generally of good quality, constitute a disadvantage, but sometimes allow mobilization of adjacent skin in a more effective way for reconstructing a three-dimensional navel.

Abdominal flaps

Borges Technique

Borges (19) proposed excision of a quadrilateral segment of skin on each side of the navel for umbilical reconstruction. The median angles are positioned approximately 1 cm away from each other at a 60° angle to create a reverse scar after suturing the excised area. The side angles are very sharp to prevent the formation of dog ears. The excised surface differs from one patient to another. This technique can be improved by adding two local lateral flaps taken from the quadrilateral segments. This technique has the disadvantage of sacrificing skin on the site to be used for reconstruction and risks creating a shallow navel with a long horizontal scar.

C-V flap technique

Shinohara et al. (20) reported an umbilical technique in which there are two V-flaps and a C-shaped flap that are sutured in a tubular manner. This technique allows for the deepening of a non-invaginated adherent scar umbilicus. The technique begins with the drawing of two lateral triangular flaps and a lower C-shaped flap. The pedicle of the three flaps is superior. The depth of the navel depends on the width of the base of the "V", and the diameter depends on the diameter of the "C". The V- and C-flaps are sutured together to create an invaginated tube. The donor sites of the V-flaps are sutured, and the rotated tubular flap is anchored in the lower midline with sutures on the package. A dressing is inserted into the reconstructed navel for about two weeks. This technique seems to be

most adapted for lean patients and children. Its main drawback is a horizontal scar outcome.

Modified "unfolded cylinder" technique

This technique, described by Ozbek et al. (21), is similar to the C-V flap technique, but has a very precise pre-operative design. It involves the inverse application of an "unfolded cylinder", which was previously described for nipple reconstruction. After delineation of the umbilical position, an "open cylinder" is drawn with a horizontal rectangle over an oval. The upper margin of the rectangle is located 1.25 cm above the midpoint. The vertical and horizontal rays of the ellipse are 2.5 and 2 cm, respectively. The width of the rectangle is as great as the thickness of the subcutaneous fatty tissue. The length of the rectangle is three times longer than the vertical radius of the ellipse (7.5 cm). The ellipse is drawn in the central third of the lower edge of the rectangle, being slightly included in the rectangle itself. The incisions follow the drawing with respect to the middle third, which constitutes the upper pedicle. The flaps are raised and defatted. Resorbable sutures are used to reconstruct the cylinder, whereas the bottom is fixed in depth with non-absorbable sutures. The donor site is sutured directly with resection of residual dog ears if necessary, and the neo-navel is filled with gauze. The advantage of this technique is that the appropriate depth of the neo-navel can be easily achieved.

Lunch box-type technique

Onishi et al. (22) described a technique of navel reconstruction using a local flap with a lower pedicle design that resembles a seed. The end of the flap is divided into two, and the two parts serve as the upper and lateral walls of the neo-navel. The umbilical depth is a function of the flap width. The whole flap is defatted and the middle surface "E" represents the bottom of the navel, whose size and shape can be freely designed. A suture is then made between points "A" and "A'" and between points "B" and "B'" for skin invagination. The flaps "C" and "D" are sutured giving the umbilicus the form of a lunch box. The superior donor site is sutured directly. A slightly compressive bandage is applied for 7 days. This technique also has similarities to the techniques described above in that the defatted umbilical bottom and two flaps make a tube. However, the lunch box-type technique may be associated with a shallow navel.

V-Y flap technique

Jamra (23) proposed an umbilical reconstruction technique using two V-shaped flaps in which one flap's base is adjacent to the apex of the other. After collection, the flaps are defatted and laterally displaced in opposite directions, and their ends are sutured to each other and the abdominal fascia. The remaining incisions are sutured after correction of the dog ears. This technique produces a deep adherence that simulates a navel, although it often results in a narrow umbilicus.

Twisted flaps technique

Yotsuyanagi et al. (24) described an umbilical reconstruction technique using two vertical rectangular flaps that are sutured to each other, tubulated and twisted with a single deep stalk. Two small lateral flaps are then laterally invaginated. The donor areas and the dog ears are sutured directly. This flap is particularly suitable for cases with medial laparotomy scars and can be performed without creating an additional scar.

Triangular flap technique

This technique, described by Pfulg et al. (25), involves creating a vertical or horizontal triangular flap drawn on an excised vertical cutaneous strip. The base of the triangle is located at the edge of the excised strip and measures about 4 cm, whereas the other two margins measure 7 and 6 cm. After excision of the adipose tissue of the umbilical reconstruction unit, the flap is folded and sutured on itself. The end of the conical flap is fixed to the abdominal fascia with resorbable 2.0 thread suture. The cutaneous excess around the neo-navel is resected and sutured vertically. A drawback of this technique is the length of the vertical scar and the wide excision that produces scars of poor quality, but neo-navels with good depth.

Iris technique

This technique was described by Miller (8). Around a circular area of missing tissue, four arciform local flaps are drawn. The flaps are symmetrical with a base of 1.5 cm and a length equivalent to a quarter of the circumference of the absent circular tissue. After mobilization, the ends of the flaps are sutured together and fixed to the abdominal wall with non-resorbable suture. The adjacent margins of the flaps are brought together so that they are rotated. The dog ears are immediately corrected. This technique creates a shallow navel and is indicated for primary or secondary reconstructions.

Umbiliconeoplasty without external scars

Double-C technique

The double-C technique was described by Illouz et al. (26) for immediate reconstruction after abdominal dermolipectomy in monobloc. It consists of drawing a circle 2 cm in diameter instead of a neo-navel. Two opposite semicircular flaps in the shape of an open C are drawn. Then, after excision of small upper and lower triangles, the two flaps are defatted, sutured to each other and fixed to the aponeurosis. A dressing based on fat gauze is maintained for 10 days. The double-C technique is simple and very useful for umbilical reconstruction in healthy skin.

Purse-string suture techniques

One example of this type of technique, described by Schoeller et al. (27) and Meirer et al. (28), is used for immediate umbilical reconstruction. Once the navel is excised, a 2 cm wide ring is drawn around the defect and defatted with scissors, preserving the subdermal vascular plexus. At the level of the deep face of the dermis at the defatted ring's outer circumference, a purse-string suture is made with non-resorbable thread. A second, shoulder, suture is made at the free dermal margin of the ring. Before tightening the two sutures, a third thread is passed that includes the skin margin at 3 and 9 o'clock and is perpendicular to the drawn line. The peripheral purse string suture is carefully pulled until the diameter of the rim decreases from 6 to 2 cm. This maneuver creates skin folds and invaginates the skin ring. The second central suture is then completely tightened and fixed to the sunrise line, imitating the bottom of the umbilical crater. The third thread is then knotted by taking a Vaseline gauze and fixing it equally on the sunrise line at the bottom of the navel.

Mateu et al. (29) described a similar technique using only one purse-string suture, but with a third defatted flap. Schwartz (30) proposed immediate umbilical reconstruction with the positioning of a purse string suture around the circular defect of the umbilicus. This suture is adjusted until the desired umbilical dimension is obtained. More sutures are then used to fix the cutaneous margins to the abdominal wall, and the bottom of the crater is left to heal by secondary intention. A drawback of this technique is that a wound persists at the end of the procedure which requires regular dressing care; an advantage is that it allows the centripetal folds and the skin tension to be decreased.

Double circle technique

The "double circle" technique described by Baack et al. (4) begins with the marking of a point on the median line of the abdomen at the top of the iliac crest. Around this point a 1 cm diameter circle and another 2.5 cm diameter circle are drawn. The skin incision is made transversely at the level of the large circle equator, preserving the smaller circle surrounded by an incised upper semicircle. The upper and lower flaps are freed to the limit of the outer circle. The inner circle of the lower flap is completely defatted and anchored with points on the deep plane to provide a large contact surface between the dermis and fascia. Then, the two upper flaps are reassembled and sutured to the band and the inner circle. Approaching points "A" and "B" creates an umbilical depression. A Vaseline gauze is placed in the depression and kept in place for 5 to 7 days. This simple technique allows one to create good umbilical adhesion, but a shallow neo-navel.

Marconi's technique

Marconi (31) has proposed a simple technique for umbilical reconstruction. The new position of the navel

is found on the abdomen in an upright position. The neo-navel is designed according to a horizontal elliptical shape of 2 × 2.5 cm. Once the peripheral incision has been made, a precise excision of the subcutaneous adipose tissue of the flap is performed, preserving the musculocutaneous perforating vessels. Subsequently, a bag suture with non-resorbable thread is made on the outer edge surrounding the "island" flap. Prolène® points fix the margins of the inner flap to the muscular plane and the outer margins. A mold dressing is placed for 4 weeks, and the sutures are removed after 2 weeks. The final scar has an elliptical appearance without an external scar. However, the defatting of the flap with preservation of the perforators is a delicate point with this technique, which entails the risk of non-negligible necrosis.

Umbiliconeoplasty with grafts

Abenavoli et al. has described the use of a full-thickness skin graft for the base of the neo-navel (32). The side walls are reconstructed using laterally based abdominal advancement flaps. Matsuo et al. have favored a graft of conchal cartilage (33). The upper wall of the navel is created by flap advancement, and the lower wall is made using a cartilage graft. The ear donor unit is closed with a rear ear flap. This technique involves the risks of failure of graft implantation and infection. The reconstructed navel has an unnatural rigidity.

Complications

Data on complications after umbilical reconstruction are lacking in the international literature. However, we have observed complications such as umbilical stenosis, umbilical or skin flap necrosis (34), scar hypertrophy and transient skin erythema. Furthermore, at times a deep neo-navel cannot be achieved, resulting in a flat umbilicus. Moreover, in some cases, patients report hypo- or insensitivity around the neo-umbilical scar.

Discussion

Umbilical reconstruction is performed in a variety of clinical situations; reconstruction may be needed in cases with lost skin, healthy skin, or cicatricial skin. Having knowledge of all the different reconstruction techniques allows one to select the most suitable technique with the best safety profile and least amount of scar potential for each case. Many techniques for reconstruction have been reported in the literature, but the goal of each is to create a natural-appearing umbilicus with a permanent and sufficient depression with minimal scarring (35, 36). Although the ideal shape of the umbilicus has been debated, recent studies have shown that the young, thin female with an attractive abdomen tends to have a small, vertically oriented umbilicus (37). The multitudinous procedures described above offer a wide range of choices; however, few authors have performed large or comparative studies, so the choice is often a personal one based on the limited experience of the surgeon. The lack of studies on umbiliconeoplasty as well as the wide variety of possible procedures suggests a lack of familiarity with this problem in many surgeons. A lack of recognition of this problem among surgeons may also play a role (38).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Physiological roles of parathyroid hormone-related protein

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Summary. *Background:* Parathyroid hormone related peptide (PTHrP) is widely expressed in a variety of normal fetal and adult tissues. *Aim of work:* Review of these normal physiologic functions of PTHrP in each of these tissues. *Method:* Performed literature search on pubmed on articles related to PTHrP and physiologic roles. *Results:* PTHrP is expressed in wide range of sites in the body with roles including relaxation of vessels and smooth muscle cells, and regulation of development. PTHrP also mediates humoral hypercalcemia of malignancy. PTHrP can be falsely elevated in benign conditions. Lastly, PTHrP has a pharmacological role in osteoporosis treatment. *Conclusions:* PTHrP has many various physiological roles besides mediating humoral hypercalcemia of malignancy. (www.actabiomedica.it)

Key words: parathyroid hormones related protein, hypercalcemia, and osteoporosis

Introduction

Hypercalcemia in patients with malignancy was initially attributed to local bone destruction by malignant cells in the 1920s. Fuller Albright, in 1941, was the first to postulate that a tumor can produce a hormone in excess that was similar to parathyroid hormone (PTH). In 1987, the development of bioassays allowed advances in identification and purification of this protein from a patient with squamous cell carcinoma of the lung who had humoral hypercalcemia of malignancy. This protein, given its structural and functional similarity to PTH, was later called parathyroid hormone-related peptide (PTHrP) (1).

The human *PTHLH* gene was subsequently mapped on the short arm of chromosome 12. The translation product of PTHrP is processed into 3 secretory forms including N-terminal, mid-region, and C-terminal regions (2). PTHrP (1-141) shares homology with PTH (1-34) at the N-terminal sequence in which the first 13 residues of the mature protein are identical. Both PTHrP and PTH bind to a common G-protein-coupled receptor called PTH receptor (PTHR1), but dissociate from the receptor by different mechanisms. The mid-region peptide is involved in placental calcium transport and also fetal skeletal development. In contrast, the C-terminal region of PTHrP, which is also called "osteostatin", activates both protein kinase C and also Src-dependent vascular endothelial growth factor receptor 2 (VEGFR2) to promote osteoblast proliferation (3).

PTHrP is expressed in a wide range of normal tissues and organs including bone, cartilage, teeth, stomach, pancreas, cardiovascular-renal system, lungs, bladder, uterus, placenta, and mammary glands whereas PTH is found exclusively in the parathyroid glands. PTHrP has paracrine and autocrine actions on receptors of many different tissues. We will review the normal physiologic functions of PTHrP in each of these tissues in detail below (summarized under table 1). Lastly, we will also discuss humoral hypercalcemia of malignancy, benign conditions with falsely elevated PTHrP, and the pharmacological role of PTHrP in osteoporosis treatment.

Table 1. Summary of the physiological roles of PTHrP by tissues and organs

Site	Proposed Roles
Bone and cartilage	• Promotes bone formation • Inhibits apoptosis of osteoblasts • Regulates chondrocyte maturation
Teeth	• Regulates dental formation and mandibular development
Stomach	• Relaxes the smooth muscle of gastric fundus
Pancreas	• Stimulates insulin production • Regulates pancreatic cell proliferation, apoptosis, and differentiation
Cardiovascular-renal system	• Vasodilator effect of smooth muscle cells in response to mechanical stretch • Heart: positive chronotropic and inotropic effects • Renal: renal vasodilation to increase renal blood flow and eGFR
Lungs	• Stimulates surfactant production • Reduces lipofibroblast to myofibroblast transdifferentiation
Bladder	• Relaxes the detrusor muscles to increase bladder compliance
Uterus	• Induces vasorelaxation in the uterine arteries
Placenta	• Stimulates placenta calcium transport
Mammary gland	• Mediates mammary developments • Secreted in milk

References: (1-23)

Physiologic roles of PTHrP by tissues and organs

Cartilage and bone

PTHrP plays an important role in endochondral bone formation and osteoblast apoptosis inhibition. PTHrP is produced by chondrocytes at the end of long bones. PTHrP stimulates chondrocyte proliferation, osteoblast formation, and subsequently bone formation (1). Mice with PTHrP deficiency died immediately after birth, in contrast, PTHrP haploinsufficient mice were born with low bone mass due to decreased bone formation and also increased osteoblast apoptosis (4). Furthermore, a proposed mechanism is that oxidative stress impairs osteoblast function by causing cellular damage and apoptosis. N- and C-terminal domains of PTHrP has been demonstrated to protect against reactive oxygen species (ROS) production by oxidative stress related agent H_2O_2 in both murine osteoblastic cells and also human osteoblastic cells (3).

Teeth

PTHrP is expressed only in the odontoblastic cells of normal dental pulp. During maturation of teeth, the activation of PTH/PTHrP receptor regulates the odontoblastic cells. In an inflamed pulp, PTHrP production is stimulated by pro-inflammatory TNF-α and IL-1β, and found in the vascular zone, pulp stroma, and odontoblastic zones. PTHrP plays an important role in angiogenesis but its function remains controversial (5)

The action of PTHrP is mediated by the p27 pathway which has a negative regulatory effect on dental formation and mandibular development. Sun et al showed that deletion of p27 enhances the dental alveolar bone formation in mice by modulating cyclin E, CDK2, Bmi-1, and antioxidant enzymes (6).

Stomach

The gastric fundus relaxes in response to the deglutition process in order to accommodate the food ingested. PTHrP is found in the gastric fundus and functions to relax the smooth muscle of the gastric fundus. PTHrP inhibits the phasic contraction by release of nitric oxide-cyclic guanosine monophosphate (NO-

cGMP) and also has a direct action on the smooth muscle by increasing cyclic adenosine monophosphate (cAMP) pathways. Both of these mechanisms promote PTHrP-induced relaxation of the gastric fundus (7).

Pancreas

PTHrP is found in the pancreatic islet and ductal cells. PTHrP acts on PTH/PTHrP receptor (PTH1P) to regulate cell proliferation, apoptosis, and differentiation. It has been demonstrated that the overexpression of PTHrP stimulates insulin production, inhibits apoptosis, and also increases pancreatic islet mass (8, 9).

Normally, PTHrP has a very low expression in the exocrine pancreas. However, PTHrP level is elevated in a mouse model of cerulein-induced acute pancreatitis that mimics human pancreatitis as characterized by acinar cell death, edema formation, and inflammatory cell infiltration.

The deletion of the PTHrP gene in acinar cells protects against pancreatitis by decreasing the release of pro-inflammatory cytokines and chemokines. These findings show PTHrP is an important mediator of inflammation and fibrosis associated with acute and chronic pancreatitis (10).

Cardiovascular-renal system

In the cardiovascular system, PTHrP is found in the smooth muscle layer of the vessel, intrarenal arterial tree, and endothelial cells. The expression of PTHrP is induced in the smooth muscle by hypertension, atherosclerosis, and after balloon angioplasty (11).

In response to mechanical stretch, smooth muscle cells produce PTHrP that functions to relax the stretched muscle by its potent vasodilator effect. Serum vasoconstrictors such as angiotensin II can also induce PTHrP mRNA and its protein. If PTHrP is overexpressed in transgenic mice, it can reduce systemic blood pressure as well as decrease vascular responsiveness (12).

PTHrP has both positive chronotropic and indirect inotropic effects when released by atrial and ventricular myocytes (13). PTHrP is also expressed in the renal arterial tree and can induce vasodilation on both afferent and efferent arterioles. Raison et al used smooth muscle specific-PTHrP knockdown to understand the physiologic effect of PTHrP in the cardiovascular-renal system. They found no effect on systemic blood pressure but decreased renal plasma flow and glomerular filtration rate (GFR). Hence, PTHrP plays a role in renal vasodilation by increasing renal blood flow and GFR (14).

Lungs

Stretch sensitive type II epithelial cells in the lungs enhance PTHrP production. PTHrP binds to PTH 1 receptors (PTHR1) to increase the expression of proteins responsible for surfactant phospholipid synthesis including PPAR-γ, leptin, and adipose differentiation related protein (ADRP). PTHrP stimulates surfactant production via a paracrine feedback loop mediated by leptin and also reduces lipofibroblast to myofibroblast transdifferentiation (15).

In particular, nitric oxide deficiency is commonly seen in many forms of pulmonary diseases. A proposed mechanism is that nitric oxide deficiency directly increases the pulmonary PTHrP expression. Subsequent activation of the pulmonary renin-angiotensin system (RAS) may also increase PTHrP expression in the lung. However, RAS may also modify pulmonary fibrosis independent of PTHrP (16).

Bladder

Relaxation of the detrusor muscle allows normal bladder storage function. PTHrP functions via the PTH/PTHrP receptor 1 (PTH1R) expressed in the detrusor muscle, renal tubules, and blood vessels in normal bladders. In response to mechanical stretch upon bladder retention, PTHrP is released from the detrusor muscle and acts as an inhibitory autocrine factor to suppress spontaneous contractions of mucosa. Thus, PTHrP may increase bladder compliance function to allow more urine storage (17). In contrast, downregulation of PTH1R may be involved in detrusor overactivity seen in end stage bladder disease (18).

Uterus

PTHrP can induce vasorelaxation in the uterine arteries which helps maximize the delivery of blood to the uterus during pregnancy. The proposed mechanism involves upregulation of PTHrP mRNA but downregulation of PTH1R mRNA expression in uterine arteries. Interestingly, the expression of PTHrP and PTH1P is shifted from the medial layer to the intimal layer of the uterine arteries during pregnancy (19). Decreased intrauterine PTHrP level is associated with growth restriction in spontaneously hypertensive rats, which suggests a role for PTHrP in maintaining placental function and fetal growth (20).

Placenta

Fetal plasma calcium levels are significantly higher than maternal calcium levels. PTH level is suppressed but PTHrP level is elevated in the fetal circulation. The mid-region of PTHrP stimulates placental calcium transport to allow calcium to be made available for mineralization of fetal skeleton. In mice with PTHrP gene deletion, fetal serum calcium was significantly reduced compared to mice with wild type PTHrP, which shows that PTHrP regulates fetal calcium homeostasis by disrupting the fetomaternal calcium gradient (21).

Mammary gland

PTHrP is expressed in epithelial cells in the embryonic mammary buds of human fetuses while PTHR1 is expressed in the mesenchymal cells. PTHrP induces the proliferation and differentiation of the mesenchymal cells. Loss of PTHR1 on mesenchymal cells causes mammary development defects. Boras-Granic et al examined mammary development in PTHrP knock-in mice that express a mutant form of PTHrP with a deletion of the C-terminus and nuclear localization signals (NLS). This mutant form of PTHrP impairs mammary mesenchyme differentiation and mammary duct outgrowth but also caused insufficient PTHrP production (22).

During lactation, PTHrP is secreted by mammary glands into the systemic circulation, which activates bone resorption and liberates skeletal calcium stores. Activation of calcium sensing receptor (CaSR) in the mammary epithelial cells during lactation downregulates PTHrP levels in the systemic circulation in response to the increased delivery of calcium. Hence, PTHrP is important in modulating the calcium concentration of breast milk (23).

Clinical Implications of PTHrP

Elevated PTHrP levels associated with malignancy

Elevated PTHrP accounts for 80% of the cases in patients with hypercalcemia of malignancy. PTHrP promotes the formation of osteoclasts to cause bone resorption but does not affect the $1,25(OH)_2D$ levels which is responsible for increased intestinal calcium absorption. Laboratory findings for PTHrP mediated hypercalcemia associated with malignancy include low PTH, high PTHrP, low phosphorus, low or normal $1,25(OH)_2D$, and variable 25(OH)D levels (24). In a large case series of PTHrP mediated hypercalcemia, solid organ malignancies made up 82.6% of the overall cases (25). Similarly, another study also found 85% of the cases are solid organ malignancies (43.4% squamous cell carcinoma, 23.4% adenocarcinoma, 15.7% hepatocellular carcinoma, and 9.6% cholangiocarcinoma) and 14.8% of the cases are hematologic malignancies (26). The results from these two case series are summarized in table 2. Lastly, PTHrP mediated hypercalcemia is associated with a poor prognosis with a median survival of less than 2 months (25).

Falsely elevated PTHrP in benign conditions

Healthy subjects have undetectable or low N-terminal and C-terminal PTHrP levels. Most clinical laboratories use an assay directed toward C-terminal PTHrP since levels of circulating C-terminal PTHrP are approximately 14 times higher than N-terminal PTHrP, making it easier to detect. Both C-terminal and N-terminal PTHrP levels are elevated in patients with hypercalcemia of malignancy.

However, the renal clearance of C-terminal PTHrP level is dependent on glomerular filtration rate. Serum C-terminal PTHrP can accumulate in pa-

Table 2. PTHrP mediated hypercalcemia associated with malignancy

Solid organ malignancy	Hematologic malignancy
• Squamous cell carcinomas (lung, esophagus, head & neck, manubrium, skin, parotid, vulva, cervix, scrotum, penis and anus) • Adenocarcinoma (cholangiocarcinoma, breast, renal cell, stomach, lung, ovary, duodenum, endometrium, and pancreas) • Hepatocellular carcinoma • Small cell lung carcinoma • Non-small cell lung carcinoma • Urothelial and bladder carcinoma • Medullary thyroid carcinoma • Neuroendocrine tumors • Myxoid sarcoma • Merkel cell carcinoma • Epithelioid hemangioendothelioma	• Multiple myeloma • Non-Hodgkin's lymphoma • Diffuse large B cell lymphoma • Acute leukemia • Chronic myeloid leukemia/small cell lymphoma • Plasma cell leukemia

Adapted from Donovan et al, Jin et al (25,26)

tients with renal failure leading to a falsely elevated PTHrP levels especially if eGFR is less than 20 ml/min. In contrast, N-terminal PTHrP level is low or undetectable in patients with renal disease. Hence, the preferred test to screen for malignancy associated hypercalcemia in patients with kidney disease is N-terminal PTHrP (27). Compared to the healthy adults, PTHrP concentration is also elevated in breastfeeding women and also in postmenopausal women with low body mass index (28).

Pharmacological role of PTHrP

Abaloparatide is a synthetic PTHrP analog with potent anabolic activity that increases bone formation while also reducing bone resorption due to the parathyroid type 1 receptor conformation binding selectivity. The phase 3 Abaloparatide Comparator Trial in Vertebral Endpoints (ACTIVE) trial found the use of subcutaneous abaloparatide over 18 months compared to placebo increased bone mineral density (BMD), and reduced the risk for new vertebral and non-vertebral fractures for postmenopausal women (>65 year old) (29). Furthermore, a post hoc analysis of a subgroup of ACTIVE trial showed abaloparatide therapy was associated with increased BMD and reduced risk for vertebral and non-vertebral fractures in post-menopausal women aged 80 years or older (30). Another double blind placebo controlled trial showed 24 weeks of abaloparatide increases the BMD of the lumbar spine, femoral, and total hip compared to the placebo group. Total hip BMD increase by abaloparatide (2.6% at 80 μg) is greater compared to the teriparatide group (0.5%) and placebo group (0.4%). There was a low incidence of abaloparatide induced hypercalcemia due to lower rates of bone resorption observed in these patients. Other adverse events were similar between all treatment groups and included dizziness, injection site reactions, headache, dyspepsia, syncope, diarrhea, arthralgia, and upper abdominal pain (31). The FDA approved abaloparatide on April 2017 for the treatment of postmenopausal women with osteoporosis. This new osteoporosis therapy is promising given its ability to increase BMD in the lumbar spine, femoral and total hip, safety data, and low incidence of hypercalcemia.

Conclusions

PTHrP is widely expressed and plays a physiological role in a variety of normal fetal and adult tissues. The insights into these roles and the proposed mechanisms come from animal studies through overexpression, mutation, or deletion of PTHrP. Further studies will be needed to elucidate the mechanisms by which PTHrP acts on these tissues. PTHrP is also an important mediator of malignancy-related hypercalcemia in a wide range of solid and hematologic malignancy which is associated a poor prognosis. In an exciting di-

rection, the FDA approved a synthetic PTHrP analog that can increase BMD and reduce the risk for vertebral and non-vertebral fractures for postmenopausal women. Understanding the physiological role and malignancy associated hypercalcemia will help us further develop other therapeutic options in the future.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Science and Religion: Enemies for life?

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Summary. The article published by Prof. Antonio Neviani in 1896 offered us an interesting opportunity to discuss about the teaching of human evolution in schools today. Already at the end of the nineteenth century, Neviani complained about the fact that the teaching of the theory of evolution was not present in schools. Here, we present the thought of Neviani and we invite to reflect on the prohibition, still present in some countries, of the teaching of Darwin's theory. (www.actabiomedica.it)

Key words: Antonio Neviani, teaching of the theory of evolution, Darwin's theory, Paleoanthropology

Science and faith: an ancient battle

It is probable that in the history of scientific thought in the last two centuries, apart from the complex and unprecedented contemporary bioethical problems, no theory has been so charged with philosophical, theological and social implications that has so deeply divided the international scientific community as the evolutionary one (1).

Together, and perhaps even more, of what has been a revolution from the scientific point of view, the Darwinian thesis has, in fact, modified the world view, including the position of man in nature, to an not inferior extent to what happened in the Copernican breakthrough.

For many aspects, the impact of this theory is even more subversive than the Copernican revolution that dethroned Earth from its presumed centrality in the universe, because its consequences directly invest the dimension of time and space which, consequently, is relativised and subjected to mere rules of historical contingency.

Therefore, it is understandable how the advancement of knowledge in fields such as genomics, epigenetics and biology of development constantly solicit new assessments of biological evolutionism. This has also involved, in addition to its scientific dimension, the anthropological one connected to the impacts, of this new interpretative code of biological reality and of the same "Human Phenomenon" (recalling a famous work by Teilhard de Chardin), on the mentality, culture, religious experience and, in different ways, also on the quality of human and social life.

In reading evolutionary dynamisms, extremist tendencies together with a rhetorical use of complexity can easily lead to relativistic results: yesterday as today.

The resistance in the past and the recent various attempts to exclude Darwinian evolutionary theory from school curricula testify to the continuing difficulty in addressing these issues objectively, free from ideological conditioning.

The analysis of some relative cases for the inclusion of the Darwinian evolutionary theory in the scholastic programs solicits some considerations in the subject of the relationship of science and faith and of the defense of a rational debate.

The case of yesterday

Previously, in an article published in 1896, the author Prof. Antonio Neviani complained about the fact

that in schools natural sciences were the least subjects taught and that the teaching of the theory of evolution was not present (2). In particular, he emphasized that it was this theory that advanced all the various types of scientific studies.

It is interesting to note that Prof. Neviani carried out a survey during those years on teaching natural sciences in the Italian high schools.

He approached all the high school professors, some of the University and some of the private school professors.

Neviani reported that among the various questions, the 21st question asked: "Is it useful to make the theory of evolution known? Within what limits?"

Only 6 professors answered no, while 76 others agreed to the teaching of the theory of evolution.

Of the 76 professors, Neviani stressed that 38 answered yes without any observation; 12 intended that the maximum development be given to this study and the basis of all the teaching be made of it; by contrast, 23 asked that they give themselves very simple notions and in the most concise way. He noticed that two, fearful of who knows what, answered yes, but added: for the animals, excluding the man. Finally, there was a professor who, after a good yes, wrote: but as a historical document.

Neviani was pleased to note that all the professors, while making some reservations, approved the teaching of the theory of evolution. In particular, he reported the observation made by one of them:

"The undersigned has no complaints against the theory of evolution, of which he sees very well if not the possibility, if not for the fact of reality ... and this theory, the writer and his colleague (also a priest) teach fluently and without any fear with some reservations, etc ... Here, he recommended that in this regard he does not suggest anti-clericalism£.

"Assuming that no scientific theory has anything to do with clericalism, or with Catholicism or any other profession of political or religious faith, I consider universal science to be so, I can not but notice that there are very few priests so tolerant; for it is sufficient, for example, for it to have been experienced by a visitor to some lent, hearing of the usual critiques against Darwin and the Darwinists being thrown from the pulpit." […].

"It is certainly not my intent to take into consideration here or even to mention only works, memories or speeches made against evolutionism. Scientific works and facts with conscience, are to be taken seriously, and they must be studied with the greatest weighting, for this reason I would go too far out of the theme that I proposed; but I can not fail to remember a speech held in Rome, where we talked about the new scientific doctrines with so little seriousness of arguments, that, if everyone were to judge by this alone, we could live quietly, [...] and not deal with anti-evolutionary theories, whatever they are (2) (Prolusion read in the solemn distribution of prizes to young people of the Gymnasium-Liceo del ven. Seminary of St. Peter's in the Vatican on January 30th, 189).

So, the distinguished professor would like a science based on Catholicism [...].

He did not approve the modern scientific direction in any way, so much that he exclaimed:

"As is the case of all the strangest and most absurd theories (these principles). They are reflected on all branches of the scholarly man, so that all disciplines more or less materialize in form and substance, and appearing in the splendor of the most recent treaties. Scientists hid their most obscene and humiliating ideas.

So, we see anthropology reduced to purely physical science; psychology is based on experiences of the nervous system, in order to know the amount of heat that develops in the brain in the formation of thought; the time that takes a feeling to be transmitted to the nerve centers ..."

He also adds

"Even linguistics, a new science that studies the slow passage from ancient to modern languages, discovers the natural link that must exist […] and solves the most important problems of philology and history. We wanted to address a false way, submitting it to the laws of dreamed organic evolution, and here comes a truly symptomatic phrase medicine itself has become atheistic and materialistic".

Neviani commented asking were these absurdities and errors of modern naturalism.

Neviani went on to say that the denial of the utility that has brought about the application of the experimental method and of the evolutionary concept, in every branch of human knowledge, is precisely how

to negate the light of the sun in the clear afternoon of a beautiful day! He also reflected on the struggle between creationists and evolutionists.

In May 1891, Huxley wrote that, even if the efforts made by the proponents of the evolution theory had produced considerable fruit during forty years, it was necessary that the younger generation did not rest on the laurels reported by those who had struggled so much and considered the battle only half won.

Today's cases

The Turkish education ministry has announced that Darwin's theory of evolution will be excluded from high school programs, starting from 2019 (3). According to the Turkish government these arguments cannot be understood by the students and therefore the training curriculum must be "simplified" so that education is in line with local and national values.

It should also be remembered how, at the time of the Ottoman Empire, "materialist" thinkers like Abdullah Cevdet and Suphi Ethem had translated several evolutionary works, including the works of Ernst Haeckel.

During the years of the nationalist republic Atatürk, the theory of evolution entered school textbooks and popular culture.

Even Italy, while not ceasing to honor the evolutionary disciplines, has not escaped fundamentalist and pseudo-religious temptations (4-6).

In the legislative decree n. 59 of 19 February 2004 National indications for personalized study plans in the Secondary School of First Degree, the teaching of evolution was omitted. The list of topics to be discussed no longer included: "The evolution of the Earth", "The appearance of life on Earth", "Structure, function and evolution of living beings", "The biological and cultural origin and evolution of the human species".

The massive protests from the scientific community, including the Accademia dei Lincei, led the Italian Minister to nominate a technical commission, presided over by the Nobel Prize winner Rita Levi-Montalcini and in February 2005, she delivered the final "report". This relationship, which established that "the study of evolution is essential for an integral vision of life" since neglecting Darwinism "would seriously damage the intellectual formation of young people, who must open themselves to the observation of reality with a critical sense" (7).

Based on these considerations, the commission asked the Ministry to recognize as soon as possible the importance of Darwin's teaching both in primary school and in both cycles of the secondary school. The teaching was then reintroduced in 2005. But, Italy is a country full of surprises. In 2006, another program review commission again took up the texts and the expressions "Darwin", "biological evolution", "human evolution" were again eliminated. Similar attempts to influence national educational policy have occurred not only in the United States, but also in Russia and in many European countries (4).

What in any advanced country is a fundamental acquisition of science and culture, as well as an indispensable framework for understanding the whole complex of life sciences, in Italy - in that period - it has become a linguistic taboo.

Also, at international level, the Council of Europe in 2007 addressed the issue of teaching creationism in schools as an alleged scientific theory (Parliamentary Assembly of the Council of Europe 2007). In discussion there was no possibility of presenting Creationism in matters such as the History of Thought or Religions, or even Cultural Anthropology or the like, but rather the opportunity to present Creationism as a scientific theory, to equal the others. After extensive discussion, the issue was put to a vote.

The plenary assembly opposed the teaching of creationism as a scientific discipline. Disregarding the inconceivable need for a vote to decide on an issue of this nature, the result achieved would seem consistent with the principles of a secular society. However, the more detailed analysis of the conduct of the vote calls for considerations worthy of interest.

There were 47 nations present, 76 parliamentarians voted; 48 denied scientific relevance to Creationism, 25 expressed their adherence to teaching, considering it a scientific discipline and 3 abstained. In Italy: 3 out of 4 voted for the opportunity to present Creationism as a scientific theory to be proposed to students on a par with the theory of Evolutionism and only 1 parliamentarian expressed himself in the op-

posite direction. The Belgians, Swiss, French, Swedish, and the English voted unanimously against the teaching of creationism as a science.

Alongside Italy's position were Poland (8) and Slovakia. Serbia, Iceland and Moldova who also voted in favor of Creationism, which unanimously voted in favor of teaching this concept, considering it to be truly scientific, on a par, if not superior to other theories.

At the moment, numerous creationist organizations are increasing their activity in many countries and this goes together with the weakening of evolutionary teaching in schools (7, 9, 10, 11).

In Israel, teachers say that many students do not even know about evolution (12).

The Israel Ministry of Education considers the evolutionary theory to be a certain fact, without dealing in any way with the seeming contradiction between this theory and the Genesis creation story.

Recently (2018) (13) the Minister for Higher Education in India stated that Darwin "was scientifically wrong" and demanded that the theory of evolution be removed from school programs because no one "has ever seen an ape turn into a human being" (14).

Nevertheless Bast et al. showed that 68.5% of the participants accepted evolution, which is very high compared with other countries of the world (15).

The teaching of evolution and creationism is controversial to many people in the United States (16-18). In addition, in some countries the inclusion of evolution in the curriculum is a recent event (19).

Discussion

The cases mentioned above, which in some way go through a wide period of time (in relation to the formulation of the evolutionary theory), address the problem of the scientific nature of the thesis of creationism and are both emblematic of the enduring complicated relationship between science and faith.

The reference to greater *complexity* and, above all, to that of compatibility despite the radical difference, can help to overcome the prejudices that derive from too superficial readings of this complex relationship (20).

In this regard Einstein's definition "Science without religion is lame, religion without science is blind" is well founded, and therefore convincing.

The courageous and, at the same time, prudent interpretative program on the possibility of a meeting (in the sense in any case of their specific autonomy) between science and religion of the French Jesuit Teilhard de Chardin deserves to be recalled with due appreciation.

He is, in fact, a profound connoisseur of evolutionary theory and at the same time a promoter within the Catholic Church of a reconciliation (in the sense of compossibility without any opposition) between science and religion, criticized the fanaticism of those positions that denied the legitimate presence of complexity and, together, of the diversity between reason and faith (21).

Furthermore, the absence of an incompatibility between evolutionism and faith is explicitly expressed in the essays entitled Wer ist das eigentlich - Gott? (München 1969) in which Joseph Ratzinger states that the pre-Darwinian idea that "every single species" is "a given of creation, that, thanks to God's creative work, exists, since the beginning of the world, alongside other species as something unique and different", is a vision that "contradicts the idea of evolution and today has become unsustainable" (22).

Ratzinger proposed to separate the two spheres: "the doctrine of evolution cannot possibly incorporate faith in creation. In this sense it can rightly indicate the idea of creation as unusable for itself: it cannot be among the positive materials to which it is bound by method".

On the other hand, however, biological evolution "must leave the question open if the problematization of faith is not legitimate and possible for itself. Starting from a certain concept of science, at most it can be seen as extrascient, but it cannot in principle prohibit any question about man addressing the question of being as such. On the contrary, these last questions will always be indispensable for the man who lives face to face with the Last and cannot be reduced to what is scientifically documentable".

The thought of Ratzinger therefore at leasts partly supports the thesis of the Jesuit Teilhard de Chardin.

The distancing of the future Benedict XVI from biblical creationism, still supported today by the Prot-

estant world and, unfortunately, even by some Catholics, is clear: "it has been eliminated by the theory of evolution. The believer must therefore accept the achievements of science and admit that the manner in which he had imagined creation, belonged to a prescientific conception of the world, which has become indefensible today".

Eminent scientists, both biologists like Kolman, and physicists like Planck, have recognized the right, so to speak, of citizenship to the faith within science. The total difference between reason and faith does not compromise either trust or authority in either one or the other. However, this orientation was anticipated by Pascal, whose scientific stature in the field of mathematics and physics is widely and universally known. In fact, he stated: "The last step of reason consists in recognizing that there are an infinite number of things that surpass it. He is very weak if he does not recognize her".

Recently, moreover, by virtue of the exceptional development of mathematical logic, scientists like Gödel have identified the impossibility of proving whether some basic statements of the mathematical system are true or incomplete (23).

Even authoritative biologics and doctors, such as Monod and Deduve, affirm the opposite conception of mere chance for the rise of life or, on the contrary, that of its finalistic character. Agnosticism is therefore present in the contemporary age as regards the possibility of having a rigorous scientific certainty of the finalistic interpretation as far as the rise of life is concerned.

Therefore, from an epistemological point of view, Kant's conception of the relationship between science and faith is derived from the necessity to subordinate reason to faith.

Like all scientific theories, the theory of evolution is based on the use of the logical-rational method: it can be publicly challenged - and indeed it is desirable and opportune that this be done - to highlight its shortcomings and increase, with a further theoretical elaboration and empirical research, its explanatory power. This, of course, provided that we operate within the rules of mathematical logic.

Even democracy itself is based on logical-rational argumentation. In fact, it allows the whole community to accept or reject solutions and choices freely adopted through a logical-rational analysis based on argumentation and public discussion. And it is always on the basis of the same rules of rational argumentation that these choices can be continually questioned and revised.

Conclusions

At the dawn of the third millennium, the relationship between science and faith continues to be the subject of debate, equal to that in which in the sixteenth and seventeenth centuries the two "greatest systems of the world", the Ptolemaic and the Copernican, were compared.

This is certainly a frontier theme, like those addressed by contemporary bioethics. Also in this case the plurality of competences and the interdisciplinary can be the real key to open the discourse on the single disciplines. Furthermore, the rational approach and the methodological rigor are the essential premises to defend the scientific evidence and the rational debate.

In both cases, it is the responsibility of academic staff to provide fundamental notions correctly and effectively, freeing them from any ideological conditioning.

Identifying and understanding the possible sociocultural barriers, the errors of the science of the past and intuitive reasoning factors that make evolution so difficult to grasp can help teachers and students deal with these existential issues in the most appropriate way (23, 24).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Anatomical study in the Western world before the Middle Ages: historical evidence

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Summary. Although modern anatomy is commonly retained to begin in the XVI century, the roots of anatomical study in the Western world may be identified beforehand. An anatomical practice was present in the Western world well before the Middle Ages, starting in ancient Greece. Hippocrates of Cos (V-IV centuries B.C.) provided descriptions of the heart and vessels, and the so-called "Hippocratic Corpus" largely deals with anatomy. Aristotle of Stagira (IV century B.C.) was one of the first well-known scholars of the past to perform dissections of animals. The anatomical interest of Aristotle contained a "physiological" background too, since he was convinced that all parts of human organisms had one or more specific functions. Galen of Pergamum (II century A.D.) was the performer of hundreds of dissections of animals, and he described a great number of anatomical parts of apes, dogs, goats and pigs. The anatomical system of Galen became a gold standard for medicine for more than a thousand years, and in the Middle Ages (V-XV centuries A.D.) the human anatomy that was taught and acquired in European universities remained based on Galenic anatomy. In conclusion, Greek-speaking scholars between the IV century B.C. and the II century A.D. set the basis for the systematic dissection of animals and the comparative investigation of animal anatomical findings. These scholars also began to study the structures of the human body, interestingly taking into account the relationship between the macroscopical morphology of observed structures and their more evident functions. (www.actabiomedica.it)

Key words: Anatomy, History of Medicine, Hippocrates of Cos, Galen of Pergamum, Medical Education, Aristotle of Stagira

Modern anatomy is commonly retained to begin in the XVI century, with subsequent major achievements in the sixteen hundreds. Nevertheless, the roots of anatomical study in the Western world may be identified well beforehand. Even before the Middle Ages an ample anatomical practice was present in the Western world, starting in ancient Greece. Not by chance does the term "Anatomy" derive from Greek and it means "cutting, dissection" (1). Precisely this word can be found in the texts of Greek philosophers, naturalists and physicians of ancient times, including Aristotle, Plato, Hippocrates, Erasistratus, Herophilus and Galen (2).

Prior to the Greek civilization, in ancient Egypt human bodies were cut and opened in a religious perspective: they had to be emptied so as to allow embalming and mummification. Even if a direct and pragmatic interest for the anatomical structures of the human body was not present as such, it was Egyptian medical knowledge, as transcribed in the Ebers and Edwin Smith papyri, that permitted Greek and Roman physicians to have access to a basic sphere of reference for frequent diseases. It also set up a number of long-lasting indications for the treatment of an array of pathological conditions, ranging from limb injuries to head wounds, even using the first rudimental "surgical" techniques (3).

Historically speaking, the study of anatomy in the Western world first developed in structured terms in ancient Greece. Interestingly, in this context, in the

V-IV centuries B.C. famous Greek artists were real masters in depicting and sculpturing human bodies, showing in particular a notable attention towards muscles and limbs. The man who is considered the father of Western medicine, namely Hippocrates of Cos (460-375 B.C.), together with his School, provided interesting descriptions of the heart and vessels (4,5). In the so-called "Hippocratic Corpus", a collection of dozens of medical writings, attributed to Hippocrates, to his contemporaneous followers, but also to physicians living in successive periods, many references to anatomy are present. "The Heart" is one of the treatises included in the Corpus and it is known to be one of the first sources in Western medicine describing great vessels, the valves of the heart and the pericardium (6).

The philosopher and scientist Aristotle of Stagira (384-322 B.C.) may be considered one of the first well-known scholars of the past to perform the dissections of animals. The anatomical interest of Aristotle also contained a "physiological" background, since he was convinced that all the parts of human organisms had one or more specific functions (7). His pupil Diocles (born in the IV century B.C.) is retained to be one of the first Greeks to have performed the dissection of human bodies, and his textbook on animal anatomy is by many regarded as the first systematic reference manual on this subject in the Western world (8).

Between the IV and the III centuries B.C., in Alexandria of Egypt, the physicians Herophilus and Erasistratus began to practice animal vivisection in a systematic way, becoming the promoters of major achievements in the evolution of the anatomical study of human beings too. Herophilus (335-280 B.C.), in particular, studied at length the brain and nerves, the ovaries and what were later called the "Fallopian" tubes, the liver and pancreas, the salivary glands and the eyes. He also investigated the human arterial pulse to formulate a diagnosis of different pathologies. Erasistratus of Ceos (305-250 B.C.), by some medical historians considered as a pioneer not only in the area of animal anatomy but also in that of animal "physiology", studied the human pulse, and more in general the blood vessels and the anatomical structure of the human heart as a pump. In consequence of the investigations of Herophilus and Erasistratus, two body systems were clearly identified at the time, the nervous one (including the brain but also the muscles) and the vascular one (including the heart and vessels). In contrast to previous periods, Greek physicians from the III century B.C. onwards began to retain the peripherical pulse directly connected with the heart, differentiating its regular beat from irregular spasms and tremors, and attributing these latter disorders to the brain and to the nervous apparatus. They therefore operated a clearly anatomical, and in part functional, distinction between at least two major human systems (9-11).

Among ancient physicians, Rufus of Ephesus (80-150 A.D.), an author of treatises on pathology and dietetics, had a primary role in the history of anatomy thanks to his practical effort in elaborating a lexicon of anatomy directed to his followers. In his fundamental text "On the names of the parts of the human body" he wrote a guide for a precise and shared nomenclature in anatomy, on the basis of his strong convictions that such an enterprise would have avoided, or at least limited, misunderstandings not only in the practice of anatomy, but in the whole of medicine (12). In approximately the same period, the physician Soranus of Ephesus (98-138 A.D.), representative leader of the methodic school of medicine, provided original information on the anatomy of the female genitalia. His original contributions in the implementation of obstetrics, contained in the fundamental text "On midwifery and the diseases of women", largely influenced the practice of midwifery till the XV century (13).

What Aristotle represented for philosophy in the Western world, Galen of Pergamum (129-216 A.D.) represented for medicine. A man of letters, a philosopher and a physician, Galen was performer of hundreds of dissections of animals, evidencing and describing a great number of anatomical parts of, among others, apes, dogs, goats, sheep and pigs. Galen considered his anatomical studies as the foundation of a global medical awareness, and he was a very skilled "sector" and a genuine researcher (14). The anatomical system of Galen, established on the basis of three connected systems, the arterial, the venous and the nervous ones, became a gold standard for anatomy, and more in general for medicine as a whole, for more than a thousand years. The human anatomy that was taught and acquired in European universities from the XII century onwards, remained profoundly based on the Galenic anatomical

system (15,16), thus influencing Western medicine for the entire Middle Ages (V-XV centuries A.D.).

The authority of the philosopher Galen was so high, and the relevance of Galenic medical mastery so ample, that only an anatomical revolution, occurring in the XVI century thanks to the Flemish Renaissance physician and anatomist Andreas Vesalius (1514-1564), was able to supersede his anatomical thought. In practice, Vesalius wrote the first modern textbook of human anatomy, divided in several volumes and complemented with many anatomical tables, containing the description of the hundreds of dissections that he had personally performed, from the time he was a student of medicine (17-19).

The anatomical study in the Western world thus begins well before the Middle Ages, as evidenced in this diachronic survey that provides historical highlights that document this. In effect, Greek-speaking scholars between the IV century B.C. and the II century A.D. set the basis for the systematic dissection of animals and the comparative investigation of animal anatomical findings. These scholars began to study the structures of the human body, also taking into account, to the extent possible for the state of the art at the time, the relationship between the macroscopical morphology of observed structures and their more evident functions.

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Secret hand gestures in paintings

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Summary. During the Renaissance period, hands were as important a focus of attention as the face was, because they were the only other visible area of the body. Hence, representation of the position of the hands became a decorative element that was almost as important as the face. Thus, given its high visibility, hand gestures in portraits and paintings have been one of the most effective ways of conveying secrets, codes and messages. From the historical and religious perspective, hand signs in visual art may provide clues about the underlying iconographical symbols. This paper will examine the eventual hidden meanings behind a peculiar hand gesture that has been widely used by several painters. (www.actabiomedica.it)

Key words: hand gesture, art, hand, painting, signs

Introduction

The art of a particular period is a suitable subject of investigation for exploring the existence of congenital deformities or medical illness based on physical depictions in drawings, paintings and sculptures or peculiar gestures and symbolic hallmarks. According to deductions made based on the artists' work, physical depictions in artwork could be an artistic convention, a hallmark of the artist's school, intentional or inadvertent depictions of a real disease that affected the sitter, or symbolic/religious messages or iconographical attributes. In particular, the meanings of secret hand signs and their hidden messages in artworks have intrigued art experts since the Renaissance.

During the Renaissance period, hands were as important a focus of attention as the face was, because they were the only other visible area of the body. Hence, representation of the position of the hands became a decorative element that was almost as important as the face. Thus, given its high visibility, hand gestures in portraits and paintings have been one of the most effective ways of conveying secrets, codes and messages (1-4). From the historical and religious perspective, hand signs in visual art may provide clues about the underlying iconographical symbols. However, because the actual intentions of the artist cannot be confirmed in the absence of historical documentation, any discussion about this topic can be only speculative (4).

There is a peculiar hand gesture that is widely used by painters of several nationalities belonging to the Renaissance, Mannerism, Baroque and later artistic movements: It is an unnatural position of one or both hands in which the third and fourth digits are held tight together, as if almost fused, resembling syndactyly, and the second and fifth fingers are separated from the central ones. So far, no one has attempted to understand the common meaning of this representation. Therefore, the goal of this investigation is to discuss the meanings of and to speculate about the reasons for the depiction of this unnatural finger position. Our investigation is specifically focused on the religious meaning and the cultural iconography or the secret message conveyed by the hand posture.

Here, we have studied this gesture in four different unnatural gesture across hundreds of portraits by more than eighty painters:

- *The hand placed on the chest*
- *The hand placed on the flank*
- *The hand pointing upward to the sky or downward to the earth in a blessing position*
- *The hand grasping an object or holding a person*.

Materials

This position can be observed in the works of several artists, including masters such as Titian, Pontormo, Bronzino, Raphal Santi, Michelangelo Buonarroti, El Greco, Raphael Mengs, Juan de Juanes, and among other painters across countries and centuries. Eight portraits constitute the subject of this review, and they were used as an explicative example, with particular focus on the hands of the models (Figure 1 to Figure 8).

Results

Based on the authors' experience in the medico-artistic field (4-11), we think that the unnatural hand position in all the portrayed subjects is most probably an artistic device or a symbolic hallmark rather than a true pathologic depiction of syndactyly, given that the occurrence rate of syndactyly is not very high and that the symbol is repeatedly used in the work of certain artists. Although the idea that this gesture may be a content-laden symbol, a secret physical (hand) sign conveying a higher meaningful idea is fascinating, the present investigation has the purpose to dismiss the gesture to an uncommon oddity.

Hypotheses and criticism about the hand gesture

The hand gesture that we systematically identified is not present in any of the art manuals, symbolism guidelines, religious books, sects, or secret societies in both the western and eastern world. Therefore, five basic hypotheses have been proposed to decipher the meaning of the hand gesture. They are discussed and dismissed below.

Figure 1. *Cosimo I de' Medici*, 1538, Jacopo Carrucci aka Pontormo, Tempera on panel, 95.6x74.9 cm, Metropolitan Museum, New York

1. Crypto-Jew (Marranos) recognition

According to this hypothesis, the gesture was a secret sign used to recognize crypto-jews each other. During the time of inquisition according to the Catholic Kings' order of 1492, Jews living in Iberian Peninsula were forced to accept or to leave Spain. Many Jews, although converted, continued to practice Judaism in secret, especially in the judería, which was the old Jewish barrio (neighbourhood) of Toledo. These last Jews were called crypto-Jews or Marranos, which means *Christianized Jew*, *swine*, or *pig* in medieval Spanish. The derogatory term was used to refer to the ritual prohibition against eating pork, which was adhered to by both Jews and Muslims. In the modern Spanish language, *Marrano* still means *pig* or *dirty*, but it is no longer commonly connoted with religious contents.

Oppenhejm in his book suggested that the hand gesture was a type of a 16th century secret sign used in

Figure 2. *Christ carrying the cross*, 1577-87, Domenikos Theotokopoulos aka El Greco, Metropolitan Museum, New York

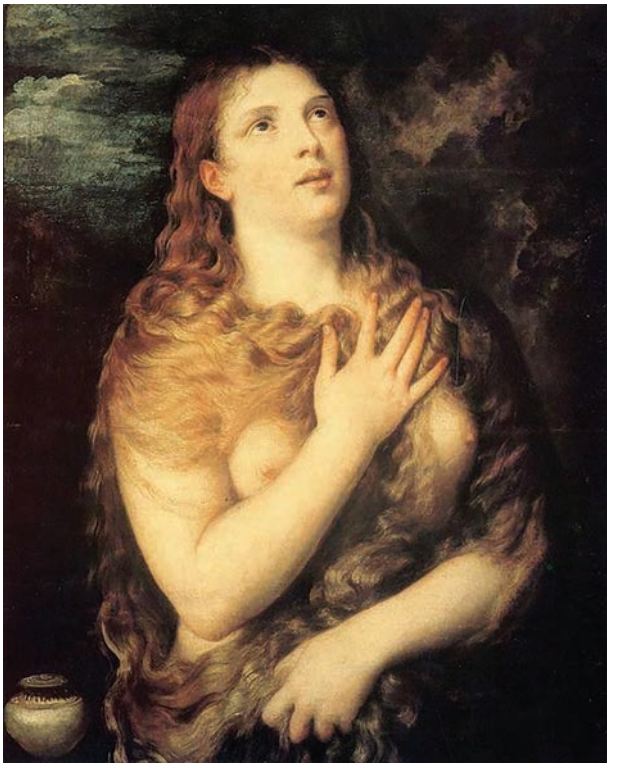

Figure 3. *Penitent Magdalene*, 1533, Titian Vecellio, oil on wood, 85x68 cm, Galleria Palatina, Rome

Figure 4. *Separation of Earth and Waters*, 1511-12, Michelangelo Buonarroti, fresco, 155x270 cm, Sistine Chapel, Rome

Toledo among the crypto-Jews (i.e. false Christians) to recognize each other (12). Although this meaning of the hand gesture may be associated with the sitters of the Spanish painter of that time, a lot of criticism has been raised because such signs/symbols are not illustrated or mentioned in any of the full-bodied Sephardic and Kabbalistic manuscripts/books from this period or their antecedents. Indeed, it would be unrealistic to assume that all the Iberian (or those who worked there) painters, such as El Greco, Luis El Divino Morales, and Juan de Juanes, were Jewish or that their patrons and sitters were Jewish. In addition, the term *Marrano* was mostly used in Toledo's judería, and not all the Spanish painters included in this investigation came from there or were working there. Therefore, it is possible that Oppenhejm confounded the gesture of the hand (herein analysed) with the well-known symbol in which the thumbs with the second and third fingers of each hand are held touching each other and separated from the fourth and the fifth fingers, conceiving a sort of fan also known as the Kohanic Blessing. This last symbol can be found carved on Jewish tombstones throughout Europe; for centuries, it has represented a cosmic symbol, and even in modern times, the gesture refers to 'an ancient matriarchal sign for strength and

Figure 5. *El caballero de la mano en el pecho (Gentleman with his Hand on his Chest)*, 1580, Domenikos Theotokopoulos aka El Greco, oil on canvas, Museo del Prado, Madrid

Figure 6. *Posthumous portrait of Christopher Columbus*, Sebastiano del Piombo, 1519, Metropolitan Museum of Art, New York. Someone consider Colombo a Jewish. [Marrano Jewish explorer, aided by Marranos Louis de Santangel and Antonio de Marchena]

power' (13, 14). Finally, there is no letter or religious gesture, Hebrew or otherwise, similar to the splayed hand. Therefore, the stylized gesture of the hand investigated in this paper is actually non-existent in the known Hebrew tradition of the 16th century, whereas the gesture of the Kohanic blessing existed for centuries prior to that period. Thus, the hypothesis of a widespread depiction of Marranos' hand recognition sign in several portraits seems inconsistent.

2. Freemasonry or Masonic membership and rank

According to this hypothesis, the gesture was a secret sign used to recognize masonic followers each other. The enigmatic posture of the hand has fascinated a lot of scholars of hidden societies, who cryptically connoted the unusual splayed fingers with the letter M, which indicated not only Masonic membership and rank, but also possession of occult secrets. The speculation that the hand gesture herein presented is a freemasonry's conveyed code is fascinating, but it is hard to accept.

3. Satanism (multiple V's and I's for 666)

According to this hypothesis, the gesture was a secret sign conveying satanic meanings. The hand gesture may be read as the letters M and W, which may be interpreted as multiple V's and I's to symbolise 666. This is because the letter V is pronounced as 'waw' in Hebrew and 'vav' in Gematria and is the 6th letter in both alphabets. However, there is no evidence to show that Renaissance, Mannerist and Baroque art had any connection with Satanism.

4. De' Medici family membership

According to this hypothesis, the gesture was a secret sign used to recognize a sitter as a member of the De' Medici family. In portraits of Cosimo and

Figure 7. *Portrait of Saint Ignatius of Loyola*, 1491-1556

his family by Bronzino, each are shown making the distinct hand gesture resembling an *M*. Some argue that this sign indicates their membership in the Medici family. However, this gesture is not uniquely depicted in members of the Medici family. In fact, it is more likely that the gesture conveyed modesty. Indeed, this gesture can be observed in the '*modest Venus*' (also known as *Venus Pudica* and later popularized as *Medici Venus*) (Figure 9), which is a 1st century BC marble copy of a an original bronze Greek sculpture depicting the Greek goddess of love Aphrodite. Some scholars argue that the gesture seen in the Medici portraits may be an allusion to this modesty. The widespread depiction of the gesture in portraits of the de Medici family created by Bronzino (Figure 8) and the paintings of artists that followed makes this speculation interesting, but it is hard to accept it as a unifying explanation.

5. Ignacio de Loyola's gesture for the atonement of sins

According to this hypothesis, the gesture was a sign with a religious meaning used during the spiritual exercises by Jesuits. San Ignacio de Loyola (Figure 7) founded the Jesuits' Order in 1541 and became its first Superior General. The Saint used to recommend a gesture for believers in his spiritual exercises: 'each time one falls into sin, in laying the hand on the breast whilst inciting one's inner self to grief.' (15). After analysing the paintings of one of the painters herein referenced (El Greco), Cassou in 1934 proposed that the painter wanted to depict the gesture with the hand placed on the chest as a sign of moral pain in sinners who were in the act of committing a sin (16). Later on, Veronica de Osa embraced the same hypothesis in her novel, *The mystic finger symbol of El Greco* (1956)

Figure 8. *Portrait of Maria de' Medici*, 1553, Agnolo di Cosimo Tori aka Bronzino, tempera on canvas, 52.5x38 cm, Galleria degli Uffizi, Florence, Italy

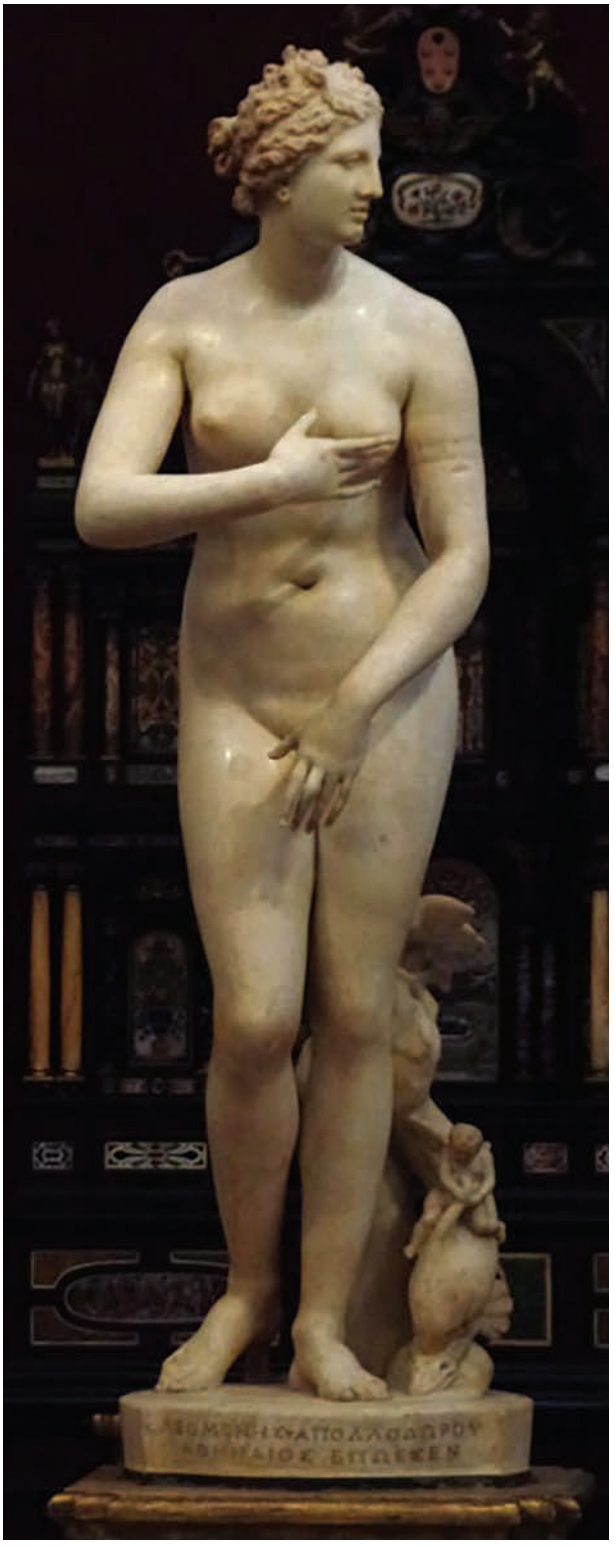

Figure 9. *Venus de' Medici* or *Medici Venus* depicting the Greek goddess of love Aphrodite. Copy of a 1st-century BC marble copy, perhaps made in Athens, of a bronze original Greek sculpture. Uffizi Gallery, Florence, Italy

(17). However, this speculation does not explain the widespread presence of the gesture in paintings. The 'Spiritual Exercises' of Loyola was published in the middle of the 16th century (1548), at which time some of the painters had already depicted their sitters with that hand gesture. It may be interesting to accept the Loyolan theory for this gesture, in the case of artists who incorporated this hand gesture after the publication of Jesuit rules, but the hypothesis is still weak.

The description of the penitent gesture by Saint Ignacio does not specify at all the position of the fingers, but it only deals with placing the hand upon the chest. In addition, Loyola warned against the display of the act of penance in public: it could be done 'even in the presence of many others but without their perceiving what the sinner is doing'. Therefore, it would be unlikely that the sitter displayed the act of penance in a painting because it was prohibited outdoors according to Loyolan laws. Further, based on the universal application of the hand gesture in the artworks of so many painters, it seems unlikely that they are trying to depict the same spiritual sense or feeling of penitence. Another speculation that goes against this hypothesis is that many painters have depicted this stylized gesture in Christ, the Virgin, and other figures of Saints, too. However, it is not likely that they would depict such a gesture symbolizing suffering and regret for sinning among non-sinning deities. Thus, it seems unrealistic to label the hand gesture herein investigated as a Loyolan content-label symbol.

Discussion

The present retrospective investigation tried to shed light on the reasons why a peculiar hand gesture resembling syndactyly was depicted in hundreds of paintings by various artists, including the great masters, such as Titian, Bronzino, El Greco, Parmigianino, François Clouet, Hans Memling, Anton Raphael Mengs and Luis el Divino Morales, who adopted this sign in dozens of portraits produced by them (Figures 1-8).

An inaccurate depiction of the model inadvertently drawn by an artist seems unlikely given their high reputation and because of the large number of paintings in which the same hand gesture has been

depicted. Indeed, the pre-Renaissance simplified and ordinary hand depictions were substituted with a more truthful and anatomically accurate hand drawing by the Renaissance artists, who captured the static and dynamic complexity of hands (4, 5). Moreover, in almost all of their works, Renaissance painters provided detailed preliminary studies through cartoons, drawings, sketches or designs, and an erroneous depiction of an abnormal finger position would not have been repeated by accident, especially as such mistakes would have been adjusted in the final piece. Further, since this gesture is found in several portraits by painters belonging to different ages, nationalities and artistic movements, and it is not limited to sitters of a particular age or family, they are most probably stylistic features of the artists' work rather than accurate depictions of anomalies in the subjects' hands. Therefore, although some of the paintings included in the present investigation show human figures with the fingers depicted as resembling syndactyly involving the third and the fourth fingers, it is most likely that the artists in their career merged attributes from various models to create an idealized beauty of the hand.

In conclusion, a lot of Renaissance and later artists are known indeed to have regularly shown finger anomalies in their paintings for stylistic reasons (4, 5); moreover, it is highly improbable that there was a veritable epidemic of syndactyly in the 15th to 17th century in Europe. Our investigation suggests instead that it was fashionable to be depicted with certain types of hand gestures, and that this feature signified grace, elegance and refinement rather than deformity. Several members of the Renaissance and later movements used this unnatural depiction of the fingers following an artistic trend of that period to imbue and to idealize the delicacy and the grace of the hand depiction. It should be considered an artistic device or a symbolic hallmark without any conveyed meaning rather than a true pathologic depiction of syndactyly.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Antidotal or protective effects of honey and chrysin, its major polyphenols, against natural and chemical toxicities

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Summary. *Objective:* Honey and its polyphenolic compounds are of main natural antioxidants that have been used in traditional medicine. The aim of this review was to identify the protective effects of honey and chrysin (a polyphenol available in honey) against the chemical and natural toxic agents. *Method:* The scientific databases such as MEDLINE, PubMed, Scopus, Web of Science and Google Scholar were searched to identify studies on the antidotal effects of honey and chrysin against toxic agents. *Results:* This study found that honey had protective activity against toxic agents-induced organ damages by modulating oxidative stress, inflammation, and apoptosis pathways. However, clinical trial studies are needed to confirm the efficacy of honey and chrysin as antidote agents in human intoxication. *Conclusion:* Honey and chrysin may be effective against toxic agents. (www.actabiomedica.it)

Key words: honey, chrysin, natural toxic agent, chemical toxic agent

1. Introduction

Nowadays, antioxidants are used for reducing risk of various diseases such as cancer, cardiovascular, neurodegenerative, renal failure, gastrointestinal, and respiratory diseases (1-3). Honey is one of the main natural antioxidants which is used as a nutritional product and an alternative treatment in traditional medicine (4). Honey is a natural liquid consisting of at least 181 ingredients including proteins, amino acids, minerals, vitamins, and organic acids (5). Honey also contains polyphenols, flavonoids, glycosides, quinone, alkaloids, cardiac glycosides, and volatile substances (6). The honey composition is related to the plant source, seasonal and environmental properties as well as honeybee species. Honey is also comprised of different impurity (7). Honey contains less fructose and glucose when compared to sugar, but contains more calories. Honey and sugar are both carbohydrates, consisting of the two types of sugar: glucose and fructose. Refined fructose, which is found in sweeteners, is metabolized by the liver and has been associated with: obesity. Although, Sugar is sugar, however, honey is (mostly) sugar. On the other hand, sugar is higher on the glycemic index (GI) than honey, meaning it raises blood sugar levels more quickly. This is due to its higher fructose content, and the absence of trace minerals. The difference between the digestion of honey compared to the digestion of sugar lies in the composition of enzymes in each of these products. Sucrose (table sugar) passes through the stomach without any digestion happening because of its disaccharide (a sugar composed of two monosaccharides) composition. This means, honey also has trace elements in it. These will depend on region, so depending on the source of your honey, it could have varying small amounts of minerals like zinc and selenium, as well as some vitamins. Where honey shines is in its content of bioactive plant compounds

and antioxidants. Various constituents of honey are found to have antioxidant activities. Flavonoids and polyphenols such as chrysin in honey indicated the strong antioxidants properties (7). The pharmacological properties of honey are associated with the presence of polyphenols in honey. Although, honey may be low in vitamins and minerals but is high in some plant compoundsincluding chrysin (7). According to the recent scientific literature, honey and chrysin may be effective against a wide range of diseases from a wound to cancer (9-13). Various studies have also reported protective effects of honey and chrysin against natural and chemical toxic agents in different tissues (14). Thus, the present study was designed to review the protective effects of honey and chrysin against tissue injuries induced by natural and chemical agents.

2. Methods

We searched the literature for basic and clinical studies concerned with the antidote and protective effects of honey and chrysin against toxic agents. The electronic databases including PubMed-Medline, Embase, Google Scholar, and Scopus were searched from 1990 to 2018. Keywords were Honey, chrysin, natural toxic agent, toxic chemical agent, and protective effects combined in different ways to retrieve related articles. After reading the abstracts, the full texts of 58 articles were critically scrutinized.

3. Neuroprotection

3.1 Honey and neurotoxic agents

Lead

Lead (Pb) is one of the major toxic heavy metals causing severe tissue injury in both human and animal. Oxidative stress has been found as an essential mechanism involved in lead-induced neurodegenerative diseases such as memory impairment and locomotor disability. Lead exposure reduces locomotor and exploratory activities and also increases anxiety and memory impairment via disrupting the oxidant-antioxidant system in animal models. The study has indicated that administration of honey (1 and 1.5 ml/kg, PO) inhibited lead induced-neurotoxicity as seen through improvement of memory and locomotor functions in animals. The study suggested that honey protected brain against lead via enhancing antioxidant activities as evidenced by increasing superoxide dismutase (SOD), glutathione-S-transferase (GST), and glutathione peroxidase (GPx) activities and glutathione (GSH) level as well as decreasing brain level of lipid peroxidation (15).

Aluminum

Aluminum (Al) is one of the most abundant heavy metals in the Earth's crust. It exists in the water, air, natural and commercial food, and medicinal agents such as drugs. Aluminum has been recognized as a neurotoxic agent inducing neurodegenerative diseases. Aluminum induces oxidative stress and increases amyloid beta level in the brain of animal models. Sub-chronic exposure to aluminum elevates lipid peroxidation and decreases GSH levels as well as catalase (CAT), SOD, and GST activities in brain. Aluminum also increases serum levels of tumor indices including arginase and a-l-fucosidase by modifying glutamate GABA system. It was reported that co-administration of $AlCl_3$ with honey syrup (500 mg/kg, PO) increased antioxidant enzymes activities and decreased lipid peroxidation level in mouse brain. Honey administration also reduced serum tumor indices levels by modulating expression of BCL-W gene which is an anticancer gene in brain cells (16).

Lipopolysaccharide

Lipopolysaccharide (LPS) causes neurodegenerative diseases by inducing inflammatory mediators in the brain of experimental models (17, 18). It is suggested that inhibition of microglia-mediated neuroinflammation is an effective strategy for treatment of neurodegenerative diseases. In this regards, Candiracci et al., 2012 (19) indicated that honey extract (0.5 and 1 μg/ml) decreased secretion of pro-inflammatory mediators such as interleukin (IL-1β) and tumor necrotic factor (TNF-α) induced by LPS in N13 microglia.

Honey decreased the inducible nitric oxide synthetize (iNOS) expressions and production of reactive oxygen species (ROS) (20). The study indicated honey may act as a potent inhibitor of microglial activation and prevent the neurodegenerative diseases (19) (Fig. 1).

3.2 Chrysin and Neurotoxic agents

Formalin

Formalin is used for inducing neuropathic pain in the animal models. Chrysin (50, 100 and 150 mg/kg, IP, 60 min before formalin injection) was effective against formalin-induced pain in rats by modulating corticosterone and noradrenaline levels in serum (21). Chrysin (5 and 10 mg/kg, IP) have indicated analgesic and anti-inflammatory effects via modulating COX-2 activity (22).

Lipopolysaccharide

Chrysin (1, 5 and 10 μM) prevented LPS-stimulated microglia cells by decreasing NO, TNF-α, IL-1β productions and expressions of iNOS and COX-2. The findings indicated chrysin ameliorated neuroinflammation through modulating c-Jun N-terminal kinase and Nuclear factor-κB (NF-κB) signaling molecules (23). Chrysin inhibited LPS-induced vascular cell adhesion molecule-1 (VCAM-1) expression in cerebral vascular endothelial (bEnd.3) mouse cells by preventing NF-κB translocation and p38 mitogen-activated protein kinase (MAPK) signaling. It seems, the neuroprotective effects of chrysin is related to its anti-inflammatory activities (24).

Streptozotocin

Streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is produced by Streptomycetes achromogenes inducing diabetes mellitus in the animal models. Brain is susceptible to hyperglycemia induced by STZ. Oxidative stress has a vital role in developing neurovascular complications in diabetes. Natural antioxidants such as chrysin can penetrate to blood-brain barrier that may be effective against diabetic neuropathy. In this context, it was reported chrysin (30 and 100 mg/kg for 26 days) combated against STZ-induced diabetes-associated cognitive decline in rats by modulating oxidative stress indices (MDA, CAT, SOD, and GSH), NF-κB, TNF-α, IL-6, IL-1β, as well as caspase-3 in cerebral cortex and hippocampus (25). Chrysin (20, 40, 80 mg/kg/day) prevented STZ-induced brain toxicity by modulating oxidative stress and decreasing MDA levels as well as an increase in total protein, SOD, CAT, and GST in rats' brain (26).

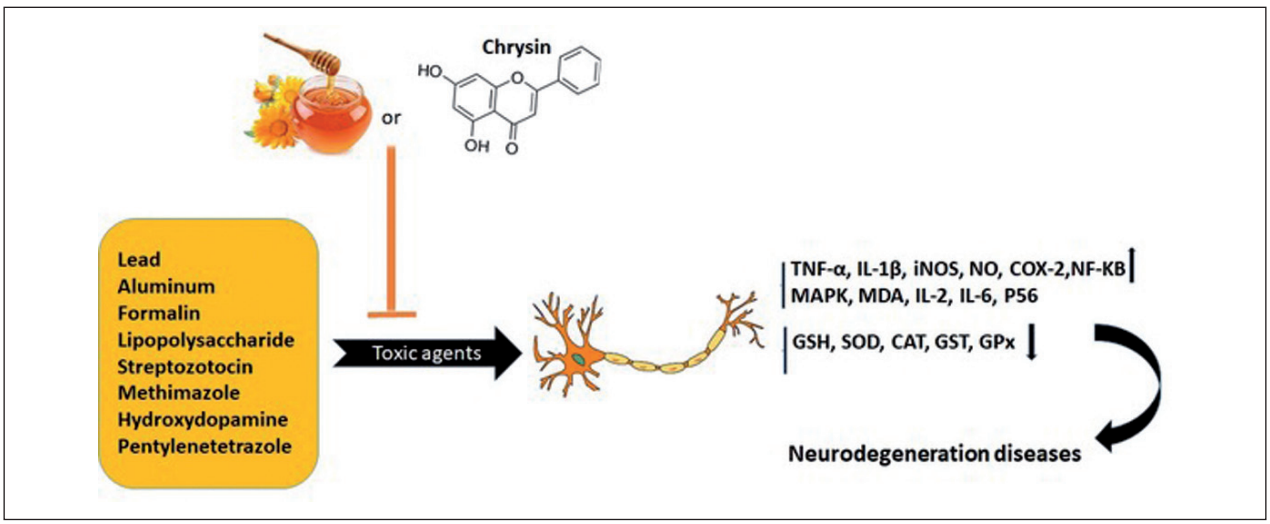

Figure 1. The protective effect of honey and chrysin against neurotoxic agents

Methimazole

Methimazole (MTZ) is useful for treatment of overactive thyroid patients. In animal models, MTZ is used for inducing hypothyroidism. Hypothyroidism usually causes depression. Chrysin (20 mg/kg, IP) represented protective effects against MTZ-induced depressive-like behavior in female mice. Chrysin modulated serotonin (5HT) in the prefrontal cortex, hippocampus as well as dopamine in hippocampus of theanimals (27).

Hydroxydopamine

6-hydroxydopamine (6-OHDA) is a neurotoxic catecholaminergic agent which is used for inducing Parkinson's disease in animal models. 6-OHDA affects inflammatory responses and neurotrophic factors in Parkinson's disease. Chrysin (10 mg/kg, PO) showed a protective effects against 6-OHDA-induced Parkinson's disease in rats. Chrysin decreased TNF-α, IL-1β, IL-2, IL-6, and NFKB levels and increased IL-10 level, total antioxidant capacity in the striatum, as well as brain-derived neurotrophic factor, nerve growth factor, and glial cell line-derived neurotrophic factor levels. Chrysin also increased dopamine, 3,4-dihydroxyphenylacetic acid, tyrosine hydroxylase, and homovanylic acid levels. Chrysin improved Parkinson's disease through modulating inflammatory cytokines and neurotrophic factors in the brain of rats with Parkinson's diseases (28).

Ammonium chloride

Hyperammonemia changes ammonia metabolism and increases inflammatory cytokines production in brain. Chrysin (100mg/kg, PO) showed protective effects on ammonium chloride (NH_4Cl)-induced neuroinflammation in rats. Chrysin up-regulated glutamine synthetase (GS) activity and glial fibrillar acidic protein (GFAP) expression as well as down-regulated TNF-α, IL-1β, IL-6, p65 NF-κB, iNOS, and COX-2 expression in hyperammonemic rats brain (29).

Pentylenetetrazole

Pentylenetetrazole (PTZ) is a selective blocker of chloride channel coupled to ɣ-aminobutyric acid type A (GABAA) receptor complex. PTZ has been used as a circulatory and respiratory stimulant drug and in convulsive therapy. However, the side effects such as uncontrolled seizure limited its use. Recently, PTZ is applied for inducting seizure in animal models. Chrysin (2.5, 5, and 10 mg/kg) representes anticonvulsant properties against PTZ-induced convulsions in rats (30) (Fig. 1).

4. Respiratory protection

4.1 Honey and respiratory toxic agents

Ovalbumin

Ovalbumin (OVA) is the main protein of egg white applying for induction of allergic reaction such as airway hyper-responsiveness in experimental models. It was reported that the aerosolized honey at a dose of (25% (v/v) and 50% (v/v) inhibited of ovalbumin (OVA)-induced asthma on airway tissues in a rabbit model. The findings indicated aerosolized honey ameliorated OVA-induced structural changes in epithelium, mucosa, and submucosal regions of airway. Honey decreased the number of airway inflammatory cells in bronchoalveolar lavage fluid and also inhibited the goblet cell hyperplasia (31). Shamshuddin et al. 2016 (32) indicated honey at a dose of 10% (v/v), 40% (v/v) and 80% (v/v) inhibited OVA-induced allergic asthma in mice model by decreasing inflammatory cell infiltration and beta-hexosaminidase level in bronchoalveolar lavage fluid. All histopathological evaluations confirmed the preventive effects of honey against OVA-induced allergic asthma. It evidenced through improving epithelium thickness, number of mast cell and mucus expression in the treated animals (32) (Fig. 2).

4.2 Chrysin and respiratory toxic agents

Ovalbumin

OVA induces lung inflammation and airway hyperresponsiveness via shifting immune response toward a T-helper type 2 (Th2) profile in mice airway. Chrysin (50 mg/kg, PO) had protective effects against

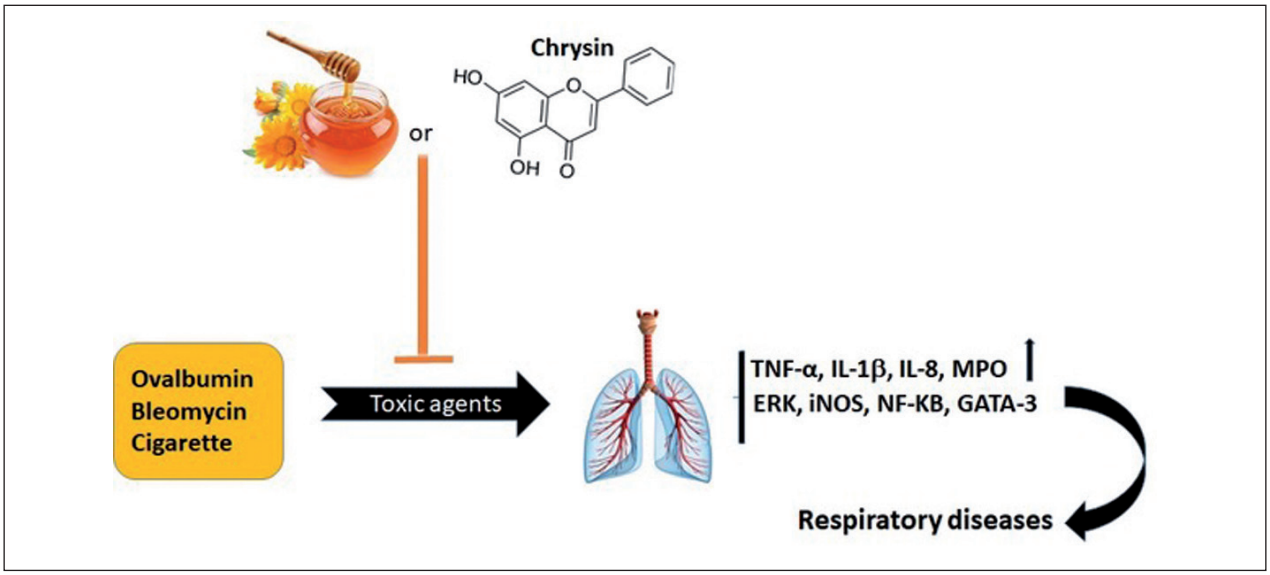

Figure 2. The protective effects of honey and chrysin against respiratory agents

ovalbumin-induced inflammation by regulating transcription factors T-bet and GATA-3 in mice (33). Chrysin (3, 10, and 30 mg/kg, PO) also ameliorated histopathological lung injury in the rats exposed to ovalbumin. It was suggested that anti-asthmatic effects of chrysin might be associated with its protective effects on Th1/Th2, iNOS and NF-κB expressions (34).

Bleomycin

Bleomycin is an anti-cancer drug inducing pulmonary inflammation and fibrosis. Bleomycin induces lung injury through producing inflammatory cytokines such as IL-18 and IL-1β in and oxidative stress (35). Chrysin (50 mg/kg, PO) indicated protective effects against bleomycin-induced lung inflammation and fibrosis in rats by modulating oxidant-antioxidant system (36).

Cigarette smoke

Cigarette smoke induces airway inflammation through increasing inflammatory cytokine in serum levels such as TNF-α, IL-1β, IL-8, and MPO. Chrysin pretreatment (10 and 20 mg/kg, IP) inhibited cigarette smoke-induced airway inflammation via modulating ERK and p38 phosphorylation (37) (Fig. 2).

5. Cardiovascular protection

5.1 Honey and cardiovascular toxic agents

Cadmium

Cadmium (Cd) is one of the major heavy metals that is associated with various diseases by inducing cellular toxicity (12). Cd produces cardiovascular abnormalities via modifying oxidative and inflammatory responses. Cd elevated mean corpuscular volume and reduced white and red blood cells counts, lymphocytes, platelets, hemoglobin, and hematocrit. However, it was reported honey (50% concentration, PO) increased hemoglobin and white blood cell count in rats exposed $CdCl_2$ (38). Abdelaziz et al. 2013 (39), also reported orally administration of honey (9 mg/kg, 1 h before a single dose of $CdCl_2$ injection) decreased in hematotoxic effects of Cd by ameliorating the changed blood parameters (39).

Lead

Some studies have indicated the hematotoxic effects of lead in animal models. It was indicated that honey (1 mg/kg, o) improved lead-induced anemia in rabbits. The study suggested the protective effects of honey against lead-induced blood toxic effects (40).

Copper sulfate

Copper sulfate ($CuSo_4$), an inorganic compound, is used as a pesticide and herbicide that its toxicity is related to copper content. Copper is an "essential mineral" that is very toxic at high concentrations. Copper causes oxidative stress by inducting Fenton-type redox reactions. However, copper toxicity usually occurs following disruption of absorption, distribution, and excretion of this metal. Copper toxicity might be associated with cardiovascular diseases. In vitro study conducted by Makedou et al., 2012 (41) have indicated that Greek pine tree honey (100, 200 and 400 µg/ml diluted serum, and 10, 20 and 40 µg/ml diluted LDL, respectively) prevented cardiotoxicity of copper sulfate by decreasing susceptibility of human LDL-c to oxidation. The study indicated Greek honey could delay LDL oxidation induced by copper sulfate, and may be useful for treating cardiovascular diseases (41).

Cigarette

Cigarette smoking plays a principal role in initiating and progression of cardiovascular diseases by inducing inflammation. Honey consumption (20 g/day, PO) ameliorated inflammatory indices including high sensitive C-reactive protein (h-CRP), IL-6, and TNF-α among chronic smokers from Quit Smoking Clinic and Health Campus, Universiti Sains Malaysia. The study suggested that honey inhibited the development of cardiovascular problems by ameliorating inflammatory response (42).

Isoproterenol

Isoproterenol is one of the non-selective β agonist drugs that are effective for treatment of bradycardia, heart block, and asthma. However, several studies have indicated the cardiotoxicity effects of isoproterenol in the animal models (43-45). Isoproterenol increased the cardiac enzymes activity including lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), aspartate transaminase (AST), alanine transaminase (ALT), as well as the cardiac troponin I (cTnI) triglycerides (TG), total cholesterol (36), low-density lipoprotein-cholesterol (LDL-C), and lipid peroxidation levels. Isoproterenol also decreased the activities of antioxidants enzymes and GSH levels in heart as well as high-density lipoprotein-cholesterol (HDL-C) serum levels. Pretreatment of Tualang honey (3 g/kg, PO) and Sundarban honey (5 g/kg, PO) in the ischemic rats inhibited isoproterenol-induced myocardial infarction through modulating oxidative stress and dyslipidemia (46, 47).

Ethanol

Clinical observation in a patient presenting with a history of alcohol abuse and experimental studies indicated the association between ethanol consumption and cardiovascular diseases. The protective effect of Anzer honey against ethanol- increased vascular permeability in rats has been studied. The results indicated that Anzer honey (0.275 g/kg, PO) with 25.44 mg/g ascorbic acid inhibited vascular permeability after ethanol exposure. It was suggested that the vascular protective effects of Anzer honey against ethanol might be related to the antioxidant content including a high amount of the ascorbic acid (48).

Lipopolysaccharide

LPS induces innate immune responses leading to the release of cellular NO and other inflammatory mediators as well as macrophage migration which causes sepsis (12). High doses of LPS is associated with organ failure and induces a systemic inflammatory response and septic shock. The effect of honey against a lethal dose of LPS-induced organ failure in rabbit has been studied. Honey (1 mL of 500 mg/kg in saline, IV) protected cardiac and lipid profiles against a lethal dose of LPS as evidenced by ameliorating red blood cell (RBC), white blood cell (WBC) and thrombocyte counts, blood pH, neutrophil infiltration and MPO activity. The study suggested that honey may be protected organs failure during inflammatory disorders (49).

High-Fat Diet

A high-fat diet is one of the leading risk factors for obesity and cardiovascular diseases distributing lipid profile. Several studies indicated that honey

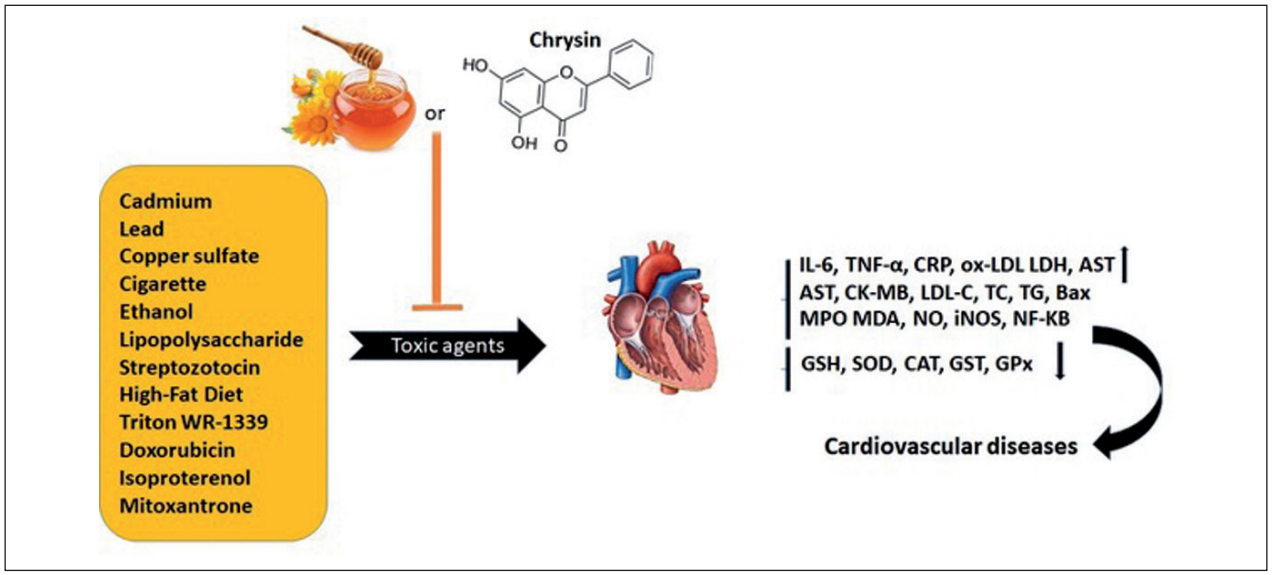

Figure 3. The protective effects of honey and chrysin against cardiotoxic agents

consumption is useful for controlling lipid hemostasis. Gelam honey decreased the percentage of adiposity index including body mass index (BMI) and the TC, TG, leptin, and resistin plasma levels in rats fed high-fat diet. The study suggested honey consumption decreased the risk of obesity and cardiovascular diseases by modulating lipid metabolism (50) (Fig. 3).

5.2 Chrysin and cardiovascular toxic agents

Triton WR-1339

Triton WR-1339 is used for inducting hypercholesterolemia in the animal models. Triton WR-1339 causes hypercholesterolemia by inhibiting lipoprotein lipase activity. Hypercholesterolemia is one the main risk factors for progressing coronary artery disease such as atherosclerosis. Chrysin (200 mg/kg, PO) improved Triton WR-1339-induced hypercholesterolemia by increasing the enzymatic and nonenzymatic antioxidants in rats (51).

Doxorubicin

Doxorubicin (DOX) is one of the anticancer chemotherapeutic drugs with severe side effects including cardiotoxicity (52). Chrysin (25 and 50 mg/kg, PO) indicated protective effects against DOX-induced acute cardiotoxicity via modulating oxidative stress indices (GSH,CAT, SOD and MDA), inflammatory markers (NF-κB, iNOS, COX-2 ,TNF-α and NO) as well as apoptotic components (Bax, Bcl-2, cytochrome c , caspase-3) (53).

Streptozotocin

Chrysin (20, 40 and 80 mg/kg, IP) inhibited hyperlipidemia induced by STZ trough modulating oxidative stress in rats liver (26).

Mitoxantrone

Methotrexate (MTX) belongs to the chemotherapeutic drugs; however, its toxic effects including cardiotoxicity limited its clinical uses. MTX causes cardiomyopathy with a reduction in left ventricular ejection fraction and heart failure (54). Chrysin inhibited MTX-induced cardiotoxicity by modulating apoptosis indices such as an increase in the Bcl-2 and also a reduction in Bax and caspase-3 expressions (55) (Fig. 3).

6. Hepatoprotective effects

6.1 Honey and hepatotoxic agents

Lead

Lead is recognized as a potent hepatotoxic agent. Several underlying mechanisms, including oxidative stress, inflammation, and apoptosis are involved in hepatotoxicity induced by lead. Lead disturbs liver metabolism, decreases liver antioxidant content and increases serum levels of liver enzymes. Carob honey (1 g/kg, PO) inhibited lead-induced hepatotoxicity in rabbits as noted by decreasing the liver enzymes serum levels (40).

Aluminum

Aluminum exposure disturbs mitochondrial energy metabolism by inducing mitochondrial oxidative stress, which leads to liver dysfunction. Honey (500 mg/kg, PO) inhibited hepatotoxicity induced by aluminum chloride ($AlCl_3$) in mice as evidenced through decreasing liver enzymes, total bilirubin, and lipid peroxidation activities. The study suggested that honey decreased the hepatotoxic effects of $AlCl_3$ by modulating oxidative stress (56).

Carbon tetrachloride

Carbon tetrachloride (CCl_4) is a hepatotoxic agent that causes liver injury by induction of ROS generation, inflammation, apoptosis and cell death. CCl_4 increases the liver enzymes serum levels including ALT, AST, ALP, and GGT. Slow or rapid intravenous injection of honey (40 and 80 mg/kg, PO) was effective against CCl_4-induced liver injury in sheep (57). It was also indicated that total food restriction with 50% honey decreased the ALT and AST serum levels in rats exposed to CCl_4 (38). Sider honey (5 g/kg, PO) ameliorated CCl_4-induced hepatotoxicity in rats as noted by decreasing in the ALT, AST, ALP, and bilirubin serum levels as well as the MDA content. Honey increased liver weight, GSH content while CCl_4 decreased these parameters. Honey improved pathological damages such as cell necrosis, vacuolar degeneration, mononuclear cellular infiltration pyknotic nuclei, and apoptotic bodies in the liver of animal exposed to CCl_4 (58). The protective effects of Saudi Sider honey (SSH) against CCl_4 induced oxidative stress and liver injury have been investigated in the rat. Administration of SSH (0.5 and 1.0 g/kg/day, PO) prior exposure to CCl_4 decreased the enzyme markers serum levels including ALT, AST, ALP, GGT and also bilirubin**.** SSH also increased total protein content and reduced malondialdehyde (MDA) levels in the rats liver exposed to CCl_4. The histopathological study of liver confirmed the hepatoprotective effects of honey which is seen by a reduction in liver lesions (59). Cheng et al., 2015 (60) have investigated the impact of buckwheat honey on CCl_4-induced liver injury in mice. Administration of buckwheat (0.22 g/10 g, PO) decreased serum lipoprotein oxidation and elevated serum oxygen radical absorbance capacity. Additionally, buckwheat honey ameliorated the ALT and AST activities that are induced by CCl_4. The hepatic levels of MDA were reduced and the antioxidant enzymes (SOD and GST) activities elevated following honey administration. The findings indicated that the hepatoprotective effects of buckwheat honey might be related to its antioxidant activity (60). Sadek et al., 2016 (61) also reported that honey inhibited CCl_4-induced liver injury via increasing phosphorylase activity in liver and decreasing carbohydrate intolerance and insulin resistance index (HOMA-IR). Moreover, the modulatory effects of honey on cytokine genes such as TNF-α and transducing growth factor beta (TGF-β) have been involved in the hepatoprotective effects of honey (61).

Acetaminophen

Acetaminophen (paracetamol or N-acetyl-p-aminophenol; APAP) belongs to analgesic drugs that its overdose is accompanied with hepatotoxicity. The hepatotoxicity of acetaminophen is initially associated with over-production of N-acetyl-p-benzoquinone imine (NAPQI) by cytochrome p450. NAPQI is eliminated by binding to GSH following exposure to therapeutic doses of acetaminophen. Overdosing of APAP decreases the cellular GSH content, permitting NAPQI to attach to the cellular proteins and cause li-

pid peroxidation (LPO), which can lead to hepatotoxicity. Acetaminophen increases the ALT, AST, ALP, GGT, total bilirubin and MDA serum levels as well as proinflammatory cytokines such as TNF-α and IL-1β levels in liver tissue. Acetaminophen overdose causes liver failure in experimental animals and humans. Pretreatment with honey inhibited acetaminophen-induced hepatotoxicity by modulating oxidative stress and inflammatory responses in rats liver. Honey (5, 10 and 20 g/kg, PO) inhibited liver injury as evidenced by a decrease in the AST and ALT activities serum as well as the Il-1β levels in the animals exposed to acetaminophen. Honey increased the GSH content and GPx activity as well as decreased MDA levels in liver. The histopathological study indicated that honey improved the liver lesions induced by acetaminophen (62). Afroz et al., 2014 (63) has shown that Sundarban honey from Bangladesh (5 g/kg, PO) inhibited acetaminophen-induced liver injury by modulating oxidative stress. A hepatic histopathological evaluation conducted by Galal et al., 2012 (62) and Gupta et al., 2016 (64) indicated the protective effects of honey against acetaminophen, noted by decreasing in congestion, necrosis, hemorrhage, distorted hepatic architecture and nuclear inclusion in the rat and mice models.

Bromobenzene

Bromobenzene is an industrial chemical that used for producing pesticides and dyes. This agent causes hepatotoxicity by converting into bromobenzene 3, 4-oxide, which produces oxidative stress and liver injury. Apis cerana honey (5, 10 and 20 g/kg, twice a day, PO) had protective effects against bromobenzene-induced hepatotoxicity in mice. Honey decreased MDA level and transforming growth factor β1 (TGF-β) expression and increased the SOD and GPx activities in the liver of mice exposed to bromobenzene (65).

Metanil-yellow

Metanil yellow is one of the mono azo dyes with high water solubility. The use of metanil yellow as a colorant agent is banded, However, in developing countries, it is still used as a colorant in several food industries such as ice-creams, sweet meat, beverages, and soft drinks. Metanil yellow causes hepatotoxicity in animal models as evidenced by an increase in the enzymes levels liver including ALT, AST, ALP and gamma-glutamyl transpeptidase (GGT). It also induces the NF-κB signaling pathways and increases the inflammatory mediators expression such as TNF-α and IL-1 β in liver. Honey (2.5 mg/kg, PO) suppressed metanil yellow-induced the NF-κB expression and TNF- α and IL-1 β levels in rats liver. The study suggested that honey has hepatoprotective activity against metanil-yellow-induced liver injuries due to its anti-inflammatory properties (66) (Table 1).

6.2 Chrysin and hepatotoxic agents

Methotrexate

MTX causes hepatotoxicity via inducing oxidative stress. Chrysin (40 and 80 mg/kg, PO) showed a protective effect against MTX-induced hepatotoxicity via increasing hepatic glutathione GSH, GPx, GR, SOD, and CAT levels and decreasing lipid peroxidation in rats (67).

Acetaminophen

Chrysin (25 and 50 mg/kg, IP) ameliorated hepatotoxicity induced by acetaminophen in rats. Chrysin decreased lipid peroxidation which led to an elevation in the antioxidant enzymes activities. Chrysin modulated inflammatory responses via elevating the TNF-α and IL-1β levels. Chrysin inhibited apoptosis and autophagy through decreasing caspase-3 activity and LC3B level. Chrysin indicated a protective effect on acetaminophen-induced hepatotoxicity by reducing oxidative stress, inflammation, apoptotic and autophagic pathways (68).

Tert-butyl hydroperoxide

Tert-butyl hydroperoxide (tBHP) causes hepatotoxicity via increasing generation of cellular ROS (69). Chrysin (5, 10 and 25 μM) improved the hepatotoxicity of tBHP by modulating ERK2/Nrf2/ARE signaling pathways in rat primary hepatocytes (70).

Table 1. Hepatoprotective effects of honey and chrysin against chemical or natural toxic agents

Results	Constituents	In vitro/ In vivo	Toxic agents	References
Decreased the serum levels of liver enzymes	Honey	Rabbit	Pb	[40]
Decreased the serum levels of liver enzymes, total bilirubin, and lipid peroxidation	Honey	Mice	Al	[56]
Decreased the serum levels of liver enzymes	Honey	Sheep	CCl4	[57]
Decreased the serum levels of ALT and AST.	Honey	Rat		[38]
Decreased the elevated serum levels ALT, AST, ALP, and bilirubin Decreased the liver levels of MDA Increased liver weight , GSH content AND total protein t Improved cell necrosis, vacuolar degeneration, mononuclear cellular infiltration pyknotic nuclei and apoptotic bodies in the liver Increased the phosphorylase activity Decreased the carbohydrate intolerance and insulin resistance index (HOMA-IR) Modulated the expression of TNF-α and TGF-β.				[58, 59, 61]
Decreased serum lipoprotein oxidation, MDA content Increased the activities of ALT , AST SOD and GST Decreased inflammatory cascade via Blockage of TNF-α-converting enzyme activity and TNF-α production	Honey Chrysin	Mice	CCL4	[60, 75]
Decreased the activities of serum AST, ALT, Il-1β and MDA content; Increased the GSH content and GPx activity; Improved the liver lesions Inhibited apoptosis and autophagy via decreasing the activity of caspase-3 and LC3B level. Decreased congestion, necrosis, hemorrhage, distorted hepatic architecture and nuclear inclusion	Honey Chrysin	Rat	Acetaminophen	[62, 63, 64] [68]
Decreased MDA level and expression of TGF-β Increased the activities of SOD and GPx	Honey	Rat	Bromobenzene	[65]
Decraesed NF- κ B expression, the liver levels of TNF- α and IL-1 β.	Honey	Rat	Metanil-yellow	[66]
Modulated the (ERK2)/Nrf2/ARE signaling pathways in hepatocytes.	Chrysin	Rat	tBHP	[70]
Decreased lipid peroxidation, XO activity; Increased GSH content, CAT, GR, SOD, GPx, GST, G6PDD and QR enzyme activities; Ameliorated expression of COX-2, iNOS, levels of NF-κB, and TNF-α.	Chrysin	Rat	Cisplatin	[72]
Modulated the activities of ADH, XO and CYP 2E1 a Decreased the levels of TBARS, lipid hydroperoxides, and conjugated dienes Increased the activity of SOD, CAT, GPx, GR, GST, levels of GSH, vitamin C, and vitamin E.	Chrysin	Rat	Ethanol	[73, 74]
Ameliorated hepatic enzyme activities, lipid peroxidation, activities of SOD, CAT, GPx, GR, GST and the levels of GSH, vitamin C, and vitamin E.	Chrysin	Rat	GalN	[76]
Modulated the oxidative stress.	Chrysin	Rat	STZ	[92]
Increasing hepatic glutathione GSH, GPx, GR, SOD, and CAT Decreased lipid peroxidation.	Chrysin	Rat	MTX	[67]

Pb: Lead; SOD: superoxide dismutase; GST: glutathione-S-transferase; GSH-Px: glutathione peroxidase; GSH: glutathione; Al: Aluminum; CAT: Catalase; IL-1β: interleukin-1β; TNF-α: tumor necrotic factorNF-κB: Nuclear factor-κB; STZ: Streptozotocin; MTX: Methotrexate; CCl4: Carbon tetrachloride; tBHP: Tert-butyl hydroperoxide; GalN: D-galactosamine

Cisplatin

Cisplatin causes hepatotoxicity through inducing oxidative stress and inflammation (71). Chrysin (25 and 50 mg/kg, PO) can improve cisplatin-caused hepatotoxicity via inhibiting oxidative stress and inflammatory responses. Chrysin decreased lipid peroxidation, xanthine oxidase (XO) activities and increased

GSH, CAT, GR, SOD, GPx, GSTlevels as well as glucose-6 phosphate dehydrogenase (G6PDD) and quinone reductase (QR) enzyme activities. Chrysin ameliorated the cyclooxygenase-2 (COX-2), iNOS expressions and NF-κB, and TNF-α levels in the liver of cisplatin-treated rats (72).

Ethanol

Excessive ethanol consumption damages to the various organs including the liver. Ethanol induces hepatotoxicity in three stages including fatty liver, alcoholic hepatitis, and cirrhosis-fibrous. Chrysin (20 and 40 mg/kg, IV) indicated the hepatoprotective effects during ethanol consumption via modulating ADH, XO, CYP 2E1 and CAT activities in rats (73). Chrysin (20 mg/kg) ameliorated ethanol-induced hepatotoxicity through decreasing the thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides levels, and conjugated dienes. Chrysin increased the SOD, CAT, GPx, GR, GST activities, and even the GSH, vitamin C, and vitamin E levels in rats liver (74).

Carbon tetrachloride

Chrysin (50mg/kg, PO) ameliorated CCl_4-induced acute liver by blocking TNF-α-converting enzyme activity and TNF-α production that leads to suppressing the inflammatory cascade (75).

D-galactosamine

D-galactosamine (GalN) is a hepatotoxic agent increasing serum hepatic enzyme activities and lipid peroxidation level in the liver. Chrysin (20, 50 and 100 mg/kg, PO) ameliorated the hepatic enzyme activities, lipid peroxidation, the antioxidant enzymes (SOD, CAT, GPx, GR, GST) activities as well as GSH, vitamin C and vitamin E levels in liver (76).

Streptozotocin

STZ causes liver toxicity by inducing oxidative stress synthesized. Chrysin (20, 40 and 80, IP) showed the hepatoprotective effects against STZ via modulating oxidative stress (26) (Table 1).

7. Nephroprotective effects

7.1 Honey and nephrotoxic agents

Acetaminophen

The overdose of acetaminophen induces kidney toxicity by disturbing oxidase isoenzymes function in kidney. Acetaminophen increases creatinine, urea, BUN levels, inflammatory mediators, and oxidative stress indices in kidney tissues. Sundarban honey (5 g/kg, PO) from Bangladesh indicated nephroprotective effects on acetaminophen via decreasing BUN, creatinine, and MDA serum levels in rats. Histopathological evaluation in kidneys also confirmed protective effects of honey against acetaminophen. The protective effect of honey may be caused by binding to acetaminophen metabolites and reducing of their affnity to cellular GSH. Thus, honey increased the GSH level and excretion of acetaminophen metabolites (63).

Cisplatin

Cisplatin causes severe nephrotoxicity in humans via inducing inflammation and oxidative stress (77). Cisplatin-induced inflammation and oxidative stress in kidney through activation of NF-κB pathways. Oxidants including hydrogen peroxide superoxide and hydroxyl radicals activate the NF-κB pathways. The nephrotoxicity of cisplatin is determined by an increase in urea, creatinine and uric acid levels in serum. Cisplatin causes significant tubulointerstitial injuries, increases α-smooth muscle actin (α-SMA), fibrogenic factors, TGF-β1, and reduces the cell proliferation index, bromodeoxyuridine (Brdu). Pretreatment with honey (500 mg/kg, PO) decreased cisplatin-induced tubular epithelial cell necrosis, infiltration of the immune component into kidney and also inflammatory cytokine and adhesion molecule expression (TNFα, IL-6, ICAM-1, MCP-1) in kidney of cisplatin-treated animals. The findings indicated that cisplatin-enhanced the expression of NF-κB that has been decreased with honey. The study has suggested that honey inhibited cisplatin-induced nephrotoxicity via suppressing the NF-κB pathway (78). Orally honey administration (100 mg/kg) reversed the increased se-

rum levels of urea, creatinine, and uric acid, and also ameliorated the histopathologic alteration. Honey showed a decrease in the α-SMA and TGF-β1 expressions and an increase in the expression of Brdu (79).

The consumption of honey (3 days before the onset of cisplatin administration) decreased cisplatin-induced nephrotoxicity in cancer patients as noted by creatinine and urea serum levels (80).

Carbon tetrachloride

Carbon tetrachloride (**CCl_4**) is recognized as a potent nephrotoxic agent inducing acute and chronic renal damage. CCl_4 increases creatinine, ALP, BUN, Uric acid and NO serum levels as an index of kidney damage. Oxidative stress has a main role in nephrotoxicity induced by CCl_4.

Sider honey (5 g/kg, PO) ameliorated CCl_4-induced nephrotoxicity in the rats as noted by a decrease in ALP, urea, uric acid, creatinine and NO serum levels and also in the MDA content in kidney. Honey increased kidney weight, GSH content while CCl_4 decreased these parameters. Honey also improved the pathological damages such as cell necrosis, intertubular infiltration and cloudy swelling in kidney of rats exposed to CCl_4 (58).

The protective effects of Saudi Sider honey (SSH) against CCl_4 induced oxidative stress, and kidney injury have been investigated in rats. Administration of SSH (0.5 and 1.0 g/kg, PO) prior exposure to CCl_4 decreased creatinine, urea, and uric acid serum levels**.** SSH increased total protein content and reduced MDA levels in kidney of rats exposed to CCl_4. The histopathological study of kidney indicated that honey reversed glomeruli, interstitial tubules, and blood vessels to the normal condition (59) (Table 2).

7.2 Chrysin and Nephrotoxic agents

Acetaminophen

Chrysin (25 and 50 mg/kg, PO) prevented nephrotoxicity in rats due to its antioxidant, anti-apoptotic and anti-inflammatory activities. Chrysin modulated acetaminophen-induced oxidative stress in the kidney by increasing the GSH level and activities of antioxidant enzymes (SOD, CAT, and GPx). Chrysin additionally decreased the levels of inflammatory markers including TNF-α, IL-1β, and IL-33. Furthermore, chrysin inhibited apoptotic tissue damage through increasing caspase-3 activity. Chrysin also ameliorated autophagic tissue injury by elevating the light chain 3B (LC3B) expression (81).

Glucose

Excess of glucose consumption causes diabetes and its complications including podocyte damage and dysfunction in human and animals. Glomerular epithelial podocytes play a leading role in modulating the glomerular filtration barrier function through their foot processes. Chrysin indicated the renoprotective effects against glucose in podocytes and mouse kidneys. Chrysin (1-20 μmol/L) inhibited glomerular podocyte apoptosis through reduction of DNA fragmentation. Chrysin decreased the Bax/Bcl-2 ratio, Apaf-1 activations and cytochrome c in renal podocytes exposed to high glucose. Chrysin (10 mg/kg, PO) ameliorated proteinuria and the abnormal alterations in glomerular ultrastructure. Chrysin showed the protective effects against glucose-induced podocyte injury by regulating ER stress (82).

Adenine

Adenine is an organic compound of purine family inducing nephrotoxicity in animal models (20). Adenine increases creatinine, urea, neutrophil gelatinase-associated lipocalin serum levels as well as *N*-Acetyl-β-D glucosaminidase activity, uremic toxin 3-indoxyl sulfate, inflammatory cytokines, and urinary albumin concentration. Adenine also increases the antioxidant content and decreases lipid peroxidation in kidney. Chrysin (10, 50 and 250 mg/kg, PO) indicated the protective effects against adenine-induced renal failure in rats via regulating peroxisome proliferator-activated receptor γ and the NF-κB signaling pathways (83).

Tetrachlorodibenzo-p-dioxin

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a polychlorinated dibenzo-p-dioxin that causes ne-

Table 2. Nephroprotective effects of honey and chrysin against chemical or natural toxic agents

Results	Constituents	In vitro/ In vivo	Toxic agents	References
Decreased the serum levels of BUN, Cr, MDA,TNF-α, IL-1β, and IL-33 Increased the serum levels of GSH, the activities of SOD, CAT, and GPx and excretion of acetaminophen metabolites.	Honey Chrysin	Rat	Acetaminophen	[63, 81]
Increasing GSH level,; Decreased; Inhibited apoptotic tissue damage by increasing caspase-3 activity and autophagic tissue injury by elevating the expression of LC3B				
Decreased the expression of NFkB.	Honey	Mice	Cisplatin	[78]
Decreased the serum levels of urea, Cr and uric acid Inhibited oxidative stress	Honey Chrysin	Rat		[79, 88]
Ameliorated the histopathologic alteration Decreased the expression of α-SMA and TGF-β1	Honey	Human		[80]
Decreased the serum levels of ALP, urea, uric acid, Cr, NO Decreased the kidney levels of MDA Increased the kidney levels of GSH Improved cell necrosis, intertubular infiltration and cloudy swelling in the kidney	Honey Chrysin	Rat	CCl4	[59, 89]
Inhibited glomerular podocyte apoptosis via reduction of DNA fragmentation, decreased the Bax/Bcl-2 ratio, Apaf-1 activation and cytochrome c in renal podocytes; Ameliorated proteinuria and abnormal alterations in glomerular Protected podocyte injury via regulating ER stress.	Chrysin	Mice	Glucose	[82]
Regulated the PPAR-γ and NF-κB signaling pathway	Chrysin	Rat	Adenine	[83]
Increased the serum levels of GSH, CAT, GPx, and SOD Decreased lipid peroxidation	Chrysin	Rat	TCDD	[85]
Increased antioxidant content in kidney tissue	Chrysin	Rat	DOX	[86]
Alleviated oxidative stress and apoptotic damage.	Chrysin	Rat	5-FU	[87]
Decreased MDA, CYP 2E1, ADH, and XO Increased GSH, Gpx, CAT, and GR Modulated the ROS production	Chrysin	Rat	Ethanol	[73]

SOD: superoxide dismutase; GST: glutathione-S-transferase; GSH-Px: glutathione peroxidase; GSH: glutathione; CAT: Catalase; IL-1β: interleukin-1β; TNF-α: tumor necrotic factor; NF-κB: Nuclear factor-κB; DOX: Doxorubicin; MTX: Methotrexate; CCl4: Carbon tetrachloride;TCDD: Tetrachlorodibenzo-p-dioxin; 5-FU: 5-Fluorouracil.

phrotoxicity (84). Chrysin (2, 20 and 50 mg/kg, PO) prevented TCDD-induced nephrotoxicity through elevating the GSH, CAT, GPx, and SOD levels and reducing lipid peroxidation (85).

Doxorubicin

Doxorubicin is associated with nephrotoxicity. This drug causes nephrotoxicity via inducing oxidative stress. Chrysin (40 and 80 mg/kg, PO) treatment attenuated nephrotoxicity induced by doxorubicin through increasing antioxidant content in kidney tissues (86).

5-Fluorouracil

5-Fluorouracil (5-FU) is an antineoplastic drug that its clinical use is limited due to its toxic effects

including renal toxicity. 5-Fluorouracil causes renal toxicity via inducing oxidative stress, activating p53, Bax, and caspase-3 and down-regulation of Bcl-$_2$ expression. Chrysin (50 and 100 mg/kg, PO) showed the protective effects against 5-FU-induced renal toxicity via ameliorating oxidative stress and apoptotic damage in rat kidney (87).

Cisplatin

Renal toxicity is one of the main side effects of cisplatin that leads to limit its usage in treatment of various cancers. Cisplatin causes renal injury via inducing ROS generation. Chrysin ameliorated cisplatin-induced renal toxicity as noted by a decrease in serotonin and BUN, lipid peroxidation serum levels and XO activity which is accompanied by an increase in antioxidant enzyme (CAT, GPx, GR, and GST) and GSH levels. Chrysin showed the protective effects against cisplatin-induced renal injury by ameliorating oxidative stress (88).

Ethanol

Chronic ethanol consumption disturbs renal functions through inducing oxidative stress. Chrysin (20 and 40 mg/kg, IP) represented the protective effects against ethanol-induced renal toxicity. Chrysin administration decreased MDA, CYP 2E1, ADH, and XO levels in kidney. Chrysin also increased GSH, Gpx, CAT, and GR levels in kidney of ethanol-treated rats. Chrysin reduced renal toxicity by modulating ROS production (73).

Carbon tetrachloride

CCl_4 disturbs renal function through increasing the iNOS expression and MDA level as well as decreasing GSH and CAT, SOD, and Gpx levels in kidney. Chrysin (200mg/kg, IP) inhibited CCL_4-induced renal toxicity by inhibiting oxidative damage in rat kidney (89) (Table 2).

8. Reproductive system protection

8.1. Honey and reproductive toxic agents

Nicotine

Nicotine is one of the major components of tobacco and cigarette. Nicotine exposure disturbs the cellular oxidant-antioxidant balance and induces organ toxicities. The reproductive system is one of the targets for nicotine toxicity. Nicotine decreases percentage of sperm motility, viability and counts as well as follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels in serum. Honey administration (1g/kg, PO) improved sperm motility, viability, counts, morphology as well as FSH, LH, and testosterone levels in rats exposed to nicotine. Additionally, honey ameliorated histopathological changes including the degenerative seminiferous tubule architecture induced by nicotine (90).

Cigarette

Cigarette smoking causes sexual dysfunctions and decreases the fertility in males. Honey (1.2 g/kg, PO) markedly elevated the percentages of achieving intromission and ejaculation and raised mating and fertility markers in the male rats exposed to cigarette (91).

Conclusion

Recent years, natural products are considered as the new strategy in treatment of various diseases due to their main activities such as antioxidant. Chemical and natural toxic agents cause toxicity in human and animal specially following chronic exposure or high doses. These toxic agents lead to toxicity in the various organs such as nervous, respiratory, cardiovascular, gastrointestinal, renal and reproductive systems. The studies have indicated natural products may be effective against toxic agents in vitro and animal experiments . One of the natural products with more pharmacological protective effects is honey. Several studies have suggested that honey and its polyphenol such as chrysin decreased neurotoxicity, lung toxicity, cardio-

toxicity, hepatotoxicity, nephrotoxicity, reproductive toxicity, genotoxicity and immunotoxicity via modulating oxidative stress, inflammation, and apoptosis in the various organs. The above findings confirmed *in vitro* and animal studies. Therefore, clinical trial studies should be done to confirm the efficacy and safety of honey and chrysin for treating intoxication in humans.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

Treatment of postoperative accessory bile duct injury by fibrin glue and balloon tamponade: a case report

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Summary. Laparoscopic cholecystectomy has become the gold standard for the treatment of symptomatic cholelithiasis. Iatrogenic bile duct injuries are still a diagnostic and therapeutic problem and their incidence increased with the introduction of laparoscopic technique. This case report documents a patient with a biliary fistula from an accessory bile duct - Lushka's duct - after routine laparoscopic cholecystectomy, unresponsive to relaparoscopy, ERCP with papillosphincterotomy, biliary stent and nosobiliary tube placement and finally treated with injection of fibrin glue and balloon tamponade through the external drain. Iatrogenic bile duct injuries remain a challenging problem, in particular when they do not communicate with central biliary tree. The detection of this fistulas is more difficult and their management should be multidisciplinary. This case presentation is to emphasize importance of correct diagnostic evaluation and timely and adequate non-surgical methods of treatment of biliary fistulas. (www.actabiomedica.it)

Key words: laparoscopic cholecystectomy, postoperative biliary fistula, Luschka's subvescical duct, balloon tamponade, fibrin glue

Introduction

Gallstone disease is one of the most common disorders of the digestive system in the Western societies. Approximately 10 to 15% of the population has gallstones. Laparoscopic cholecystectomy is considered the gold standard surgical procedure (1) and it is associated with a two-to-four times higher risk of bile duct injury than open cholecystectomy. Bile leak remains a significant cause of morbidity for patients undergoing this procedure and it occurs in 0.2-2% of cases. Injury to the mid-common hepatic duct and to the accessory bile duct - Lushka's duct - are the first and the second causes of bile leaks respectively (5, 6).

The clinical presentation of biliary fistulas may vary. Factors associated with this variability include the volume and the distribution of bile in the peritoneal cavity, presence of sterile or infected bile and presence or absence of drainage. A minority of patients will be asymptomatic.

We now report a case of post-cholecystectomy bile leakage from a duct of Lushka in a 45-year-old female patient who presented persistent dull abdominal pain 7 days after laparoscopic cholecystectomy.

Case report

A forty-five-year-old female patient was admitted to our Clinic for symptomatic gallstones and laparoscopic cholecystectomy was performed without any drainage placement. On the second post- operative day the patient had abdominal pain on the right side; ultrasound showed a small subhepatic fluid collection

compatible with laparoscopic surgery. Next day the patient was discharged without any problem.

Because of severe abdominal pain, the seventh postoperative day the patient referred to our Clinic.

On admission laboratory tests were performed and showed slightly elevated values of liver transaminase and alkaline phosphatase. Ultrasound identified minor subhepatic fluid collection. On the third day after admission the patient presented increase in body temperature of 38 degrees. Abdominal computer tomography revealed abundant free fluid in all the recesses, and in particular sub-hepatic, so a biliary fistula was suspected. The same day urgent laparoscopic revision was decided and intraabdominal bile collection with biliary peritonitis was found. After careful washing and drainage placement, an endoscopic retrograde cholangiopancreatography (ERCP) showed contrast spreading from the cystic duct. Sphincterotomy (PST) and 10-French endobiliary stent placement were performed.

In the course of the next few days, the patient was treated with antibiotic therapy and was pain free. After three days she developed fever and the amount of drainage output increased. Computer tomography revealed abdominal free fluid and two subhepatic and perigastric collection, respectively 69x31 mm and 61x27 mm. A laparoscopic peritoneal toilette was performed and four drainages were placed.

During the following days, bile flow through the external drainages did not decreased in spite of fasting; an ERCP allowed visualization of contrast spreading upstream of the stent (Figure 1). After its removal a contrast spreading from the cystic duct was noticed too (Figure 2). A nasobiliary tube was placed at the biliary bifurcation.

For the detection of the leak, HIDA scintigraphy was performed but it did not showed extravasation of radionuclide. Furthermore, magnetic resonance cholangiopancreatography (MRCP) described several bile collections, the largest in perigastric region, and the bile leak from the cystic duct, near to an external drainage.

Due to the radiological finding, compatible with a cystic duct leak without any other bile spreading, an ultrasound-guided drainage of the major collection was performed.

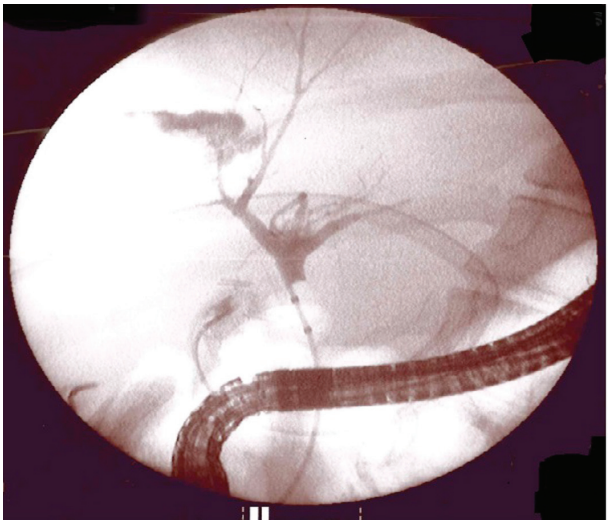

Figure 1. ERCP: contrast spreading upstream of the stent

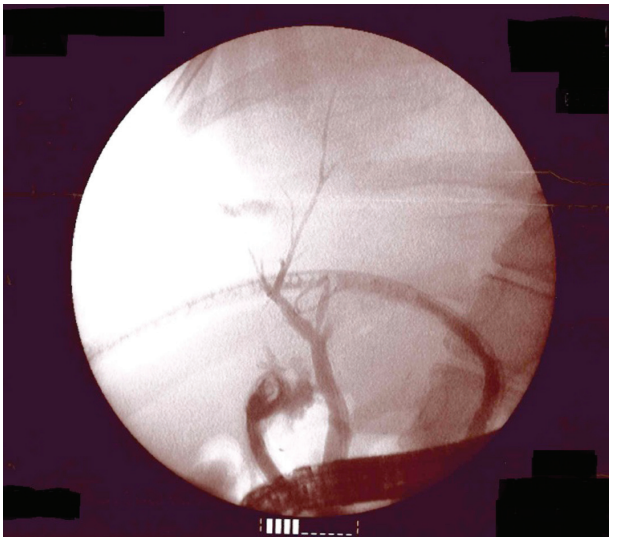

Figure 2. ERCP: contrast spreading from the cystic duct

Contrast injection through the nasobiliary tube showed spreading from the cystic duct so an ERCP was performed, nasobiliary tube was removed and a 30-French metal self-expanding stent was placed.

Even if the patient presented decrease in body temperature, the drainage output remained constant in the course of the next days both fasting and feeding so a check-up with ERCP was performed; no contrast spreading was detected. A nasobiliary tube was re-placed.

Control abdominal ultrasound described reduction of the size of the bile collections. Fifteen day after metal stent placement, this was removed, and the nasobiliary tube too; no contrast spreading was identified, neither from the cystic duct or from secondary ducts, even with injection under pressure.

Five days after the patient was discharge; a subhepatic drainage was still in place and the bile flow through this was 100 ml/day.

One month later the patient had still constant drainage output; she was readmitted to our Clinic and ERCP was performed. Intra- and extra-hepatic biliary ducts were regular, without dilatation nor filling defects; there were no contrast spreading after injection under pression. A Fogarty catheter was placed through the external drainage and a fistulography was performed by contrast injection through it, without any visualization of the biliary tree. Fibrin glue (4 cc) was injected through the Fogarty catheter, the balloon was inflated and it was left in place.

Three days after the manovre, biliary spilling was noticed and it was caused by the reduction of the balloon expansion so it was re-inflated, after fibrin glue application. No biliary loss was further detected. The patient was discharged and one week later both the drainage and the catheter were remove without any further problem.

Discussion

Postoperative biliary fistulas, if improperly treated, can lead to high morbidity and mortality. The ducts of Lushka, or subvescical ducts, are small biliary ducts which originate from the right hepatic lobe, course along the gallbladder fossa, and usually drain in the extrahepatic bile ducts. They occur in 20-50% of the population (8-10). Subvescical duct leaks follow those of the cystic duct as the most common cause of postcholecystectomy bile leaks. The incidence of this particular type of injury has not decreased despite the large collective experience in laparoscopic cholecystectomy as it might be expected; on the contrary, injuries to these ducts have occurred more frequently in laparoscopic era. The preoperative diagnosis (acute cholecystitis vs symptomatic cholelitiasis) nor the surgical setting (elective vs urgent) seems to predispose to subvescical duct injury (6).

The amount of bile that extravasates after subvescical duct injury is variable. If the injured duct does not communicate with the central biliary tree, the leak is usually self-limiting and small in volume. When it communicates with the extrahepatic bile ducts, the volume of extravasated bile may be larger. The volume of bile can range from 100 ml to more than 2 l (11-13). All factors that increase intra-ductal pressure may pose an additional problem and perpetuate a subvescical duct leak, such as coledocolithiasis or sphincter of Oddi spasm or biliary stent, like in this case (6, 9). Direct visualization of injured subvescical ducts was reported during the era of open cholecystectomy; in most cases the time of presentation of such leaks is within the first postoperative week and in a minority of cases leaks present several weeks after surgery (7).

When a postoperative biliary fistula is suspected an abdominal ultrasound and CT scan are indicated. If a fluid collection is observed, this should be drained under radiologic guidance. If the collection contains bile, an external drainage catheter should be placed.

Fistulography is one of the simplest methods to identify a biliary leak, by retrograde instillation of contrast through a drain. This is performed to demonstrate communication with the biliary tree. Several authors maintain that this should be the initial study performed in suspected cases (6). HIDA scintigraphy is a dynamic study in which an ongoing bile leak may be detected. However, it provides suboptimal anatomic detail. A subvescical duct injury will be shown as extravasation of radionuclide from the gallbladder fossa.

Anatomic details of the biliary tree with functional evaluation of biliary secretion can be obtained with a magnetic resonance cholangiogram (MRC). In addition, it can detect leaks that not communicate with the central biliary tree.

Endoscopic retrograde cholangiopancreatography (ERCP) is the most commonly used modality in which subvescical duct leaks are diagnosed. It can also provide a therapeutic solution with sphincterotomy, endobiliary stent or nasobiliary tube placement, that will lower the biliary tree pressure gradient with preferential bile flow through the papilla. Detection of bile duct leaks with ERCP mandates that these communi-

cate with the central biliary tree.

Reoperation and, specifically, relaparoscopy, can be performed in patients with severe symptoms and in those in which leakage continues despite endoscopic treatment. If an injured subvescical duct is noted, ligation should be performed.

In literature has been reported a case of resolution of a subvescical duct leak with intraoperative application of fibrin glue to the gallbladder fossa during relaparoscopy. Fibrin glue is a biodegradable, absorbable biological agent, which is widely used in surgery to reduce leakage and bleeding from the wound, promote wound healing and prevent adhesion. In the case that we have reported a fogarty catheter was inserted in the external drain and fibrin glue was instilled through it till the gallbladder fossa. The fogarty balloon was left in place to keep fibrin glue in contact with the gallbladder fossa and to tamponade the injured subvescical duct.

In literature there are no cases of treatment of bile leaks with extraductal balloon tamponade reported, while occlusion balloon (OB) is used in association with percutaneous transhepatic biliary drainage (PTBD) and it is placed through it in the biliary tree to block the biliary flow (2). The aim of the balloon is to avoid contact between bile and the fistula and to promote the healing process by shortening the healing time in the fistula.

Cozzaglio L. et al. (3) reported six cases of management of duodenal fistula after gastrectomy. The bile ducts were punctured using right intercostal percutaneous approach under fluoroscopic or ultrasonographic guidance. Over the first guide, a balloon specifically designed for vessel occlusion was positioned in the common bile duct between the confluence of the cystic duct and the sphincter of Oddi and it was manually inflated to the size deemed sufficient to stop the bile flow. Later, a drainage catheter was positioned above the balloon in order to obtain complete external drainage of bile. The catheter was left to gravity drainage. When fistula was clinically healing, a cholangiography was performed before PTBD-OB and surgical drainage removal. In case of displacement or obstruction of the drain, or deflation of the OB with the persistence of a biliary leak, patients underwent a new procedure to replace the device or reinflate the OB. They showed that PTBD-OB can stay in situ for a long time without any medical problems and it can be an effective alternative to surgery.

In literature some cases of balloon tamponade in penetrating liver lesions are also reported. (4) This method was used with good results as a rapid and easy procedure to manage hepatic transfixing lesions in abdominal trauma.

Conclusions

The use of fibrin glue in association with tamponade balloon is a feasible and safe procedure and could represent a new option in the treatment of persistent biliary leaks (1), in particular in subvescical duct fistulas that do not communicate with central biliary tree.

Further studies should be done to explore indications and timing of fibrin glue and tamponade balloon placement. In fact, better results can be expected if they are placed before the clinical situation gets critical, but not too closer the onset of the leak; in this way it is possible to exploit the formation of adhesions which bar the biliary collection in order to prevent the development of intraabdominal sepsis due to biliary peritonitis.

The use of fibrin glue alone seems to be less effective than its use associated with balloon tamponade placement.

Whatever approach, endoscopic, percutaneous or surgical, is performed, the drainage of extravased bile is mandatory as first treatment (6).

Conflict of interest: None to declare

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Case report

Management of intersection syndrome in professional motorcycle rider: a case report

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Summary. *Background and aim of the work:* Intersection syndrome is an uncommon and underdiagnosed condition, especially found in sport overuse. This case report describes the treatment of a professional motor biker who presented intense wrist pain and swelling after training sessions. *Methods:* The athlete was treated with rest, the use of a wrist splint combined with NSAIDs, physical therapy, including pulsed Nd-YAG laser therapy and exercise, such as stretching and release, massage and eccentric training. *Results:* The applied therapeutic protocol was successful and allowed the biker to completely resolve the symptoms and return to training session and professional races at full power. The results are durable at 5 months post injury follow up. *Conclusions:* This experience suggests that pulsed Nd-YAG laser therapy can be an additional useful tool in the global approach treatment for intersection syndrome, contributing with splint and manual therapy, to full recovery even in mechanically stressful conditions. (www.actabiomedica.it)

Key words: intersection syndrome, wrist pain, pulsed Nd-YAG laser therapy, tendons

Introduction

Intersection syndrome is a relatively uncommon overuse inflammatory syndrome which is often undiagnosed (1). This condition is associated with repeated radial deviation of the wrist and has been identified with a number of different names peritendinitis crepitans, bugaboo forearm, abductor pollicis longus bursitis, crossover syndrome, Oarsman's wrist, adventitial bursitis, subcutaneous perimyositis (2).

Intersection syndrome is a tendinitis with unclear pathophysiology affecting the first and second dorsal compartment. This condition is associated with the frequent and repetitive use of the wrist and it is more common in athletes (3). Possible explanations of the origin of this condition are related to the inflammation caused by friction between the extensor carpi radialis longus and extensor carpi radialis brevis where they cross under abductor pollicis longus (APL) and extensor pollicis brevis (EPB) muscle bellies in the second dorsal compartment approximately (2). Another hypothesis is related to the narrowing of the sheaths of the extensor carpi radialis lungus and the extensor carpi radialis brevis tendons, leading to swelling and pain.

Differential diagnosis includes mainly De Quervain syndrome but also tenosynovitis of other extensor tendons, blunt local trauma, or entrapment of the dorsal radial sensory nerve, ganglion cysts, infections, sprains involving the ligaments of the wrist, muscle strains, soft-tissue tumors (4).

In most of the cases, intersection syndrome responds favorably to conservative treatment, non-responders have to undergo surgery (5) to decompress the cross-over point via tenosynovectomy or fasciotomy of the abductor pollicis lungus (6). Examples of conservative therapy which have been used to treat this condition are rest (2, 4), application of ice, NSAIDs (7), wrist splinting (8), corticosteroid injection, etc.

Prevalence in the general population, varies between 0.20% and 0.37%.

High-intensity laser therapy (HILT) by pulsed Nd:YAG laser is used in physical therapy and rehabilitation. Clinical studies and scientific literature support the anti-inflammatory, anti-edematous, pain relief, and analgesic effects of Nd:YAG laser. Examples of successful clinical use of this therapy range from subacromial impingement syndrome to cervical pain (9, 10).

We often use pulsed Nd-YAG laser therapy for the treatment of tendonitis and muscle contractures in professional bikers with positive results in terms of pain management and patient acceptance. In this case report the specific use of pulsed Nd-YAG laser therapy, along with manual therapy, was carried out to solve an insertion syndrome in a professional biker.

Case report

Patient description: 22 years old Moto2 professional biker (weight: 69 Kg, height: 183 cm). His standard training activities include Flat Track, Motocross, Motard and gym exercise.

During a Flat Track training session, the biker started perceiving a mild pain at the left forearm. He reported that the circuit was quite difficult and stabilizing the bike required a large effort. During the same evening, pain intensity had a peak, therefore the biker decided to have 2 days of rest. After the rest, the painful symptoms disappeared, but as soon as the sport activity started again, the pain returned. Specifically, the pilot run 50 laps and then stopped for lunch, complaining about stinging pain. During the afternoon training, swelling of the forearm started. The biker decided to discontinue the training session and immediately proceed with a manual therapy session on site. The next day, pain was so intense that the biker was able to complete 5 laps only. The personal driving style, based on upper limb use mainly, has never caused any problem up to that moment in the biker's career.

The intersection syndrome diagnosis has been based on the clinical assessment of the patient and ultrasounds images (Fig. 1), reporting a minimal reaction of edematous type of the extensors of the periskeletal muscle sleeve. Doppler image (data not shown) shows the inflammatory reaction. Patient complained about stinging pain at the left forearm with peaks in stressful conditions, but present daily and persistent in days of maximum acuity. The clinical examination revealed pain in the following situations: palpation of the forearm radial surface, at 4 cm proximally to the radial styloid; thumb mobilization; wrist radial extension. Swelling and crepitus to mobilization of wrist and thumb were present in the same area. In days of maximum pain, this was perceived all day long, while proceeding with therapy, pain was only perceived in stressful conditions. The specific feature of this clinical case is related to the fact that once treatment has started, pain was present only under stressful conditions, which is quite unusual in tendon pathologies that are typically symptomatic until complete healing.

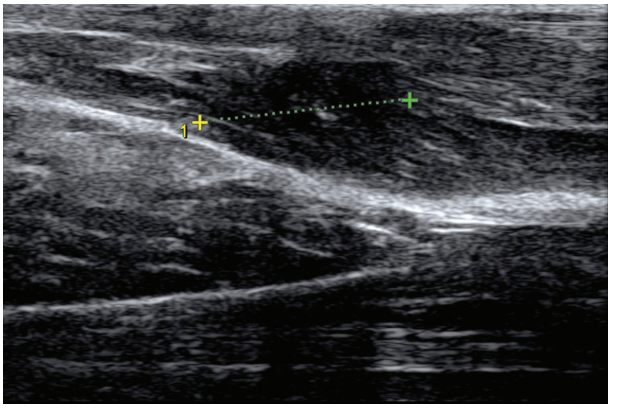

Figure 1. Ultrasound image revealing minimal reaction of edematous type of the extensors of the periskeletal muscle sleeve

Counselling has been provided to the biker in order to explain most common causes of tenosynovitis and potentially avoid future re-occurrence. The first treatment step involved rest and the use of a splint including wrist in extension and the first finger. Initially, NSAID use was prescribed daily and then, in parallel to symptoms remission, upon need. The patient had not been fully compliant with the complete rest prescription and the splint wearing instruction, using the device discontinuously.

The patient underwent pulsed Nd:YAG laser treatment (Hilterapia®, ASA Srl, Arcugnano, Italy). The technical characteristic of Hilterapia® are the following: pulsed emission (1,064 nm), brief duration

(120-150 μs), low frequency (10-40 Hz), duty cycle of about 0.1%.

The laser probe was applied perpendicularly to and in slight contact with the skin. The total energy delivered to the patient during the sessions was on average 1600J in three phases of treatment.

The first phase involved fast manual scanning over the interested forearm. An average total energy dose of 600J was administered in this phase.

The second phase involved application to the trigger points and the surrounding area. An average total energy dose of 400J was administered in this phase.

The third phase involved slow manual scanning over the interested forearm. An average total energy dose of 600J was administered in this phase.

Seven pulsed Nd-YAG laser therapy sessions were performed in a period of 8 weeks. Due to the specific Grand Prix schedule, the treatments were performed during 3 race weekends and had 4 week breaks in between. Two treatments were performed in the first two race weekends, three treatments were performed during the third and last weekend. Stretching and release of the involved muscles were carried out during the session. Each laser treatment was associated to manual therapy, such as massage and eccentric training.

All laser applications were performed by the same physiotherapist.

Given that once treatment has started, at rest no painful sensation was reported by the subject, actual pain improvement evaluation was to be postponed until the patient next bike training or exercise. This patient feedback was important to follow up patient progress in between treatments.

The clinical results that have been obtained were considered optimal by the patients. In fact, after 8 weeks from treatment start, pain has completely disappeared, even under stressful conditions, and the biker has been able to come back to usual training program and competition. Swelling and crepitus have fully disappeared. Complete wrist and thumb active range of motion (AROM) has been recovered without pain, even against resistance.

At 5-month follow up, the results are durable, especially during sport activities requiring tendon involvement.

Discussion

In intersection syndrome, pain is the key factor, along with swelling and crepitus upon palpation and/or movement. In the reported case, the patient was unable to carry out his professional sport activity due to the increasing pain that he was experiencing. At the same time, the patient needed to achieve complete pain remission as quickly as possible in order to proceed with scheduled races. The treatment approach started with patient education, discussing with the biker the details of the condition and potential etiological factors. Therapeutic plan has been based on the combination of different strategies including traditional tools such as rest, which anyway was only providing temporary relief, and the use of a splint, which obtained limited patient compliance. The patient was suggested to take NSAIDs to manage acute pain which was persistent in the first phase of the treatment. The use of rest, ice and NSAIDs represent the most common conservative approach to intersection syndrome (4, 5) and it is common to the management of most overuse syndromes. Symptoms resolve within 2 to 3 weeks for 60% of patients with rest, administration of nonsteroidal anti-inflammatory drugs, and splinting. Physical therapies including manual therapy and high intensity laser therapy were added in order to improve healing time. In many cases, the alternative to avoid surgical procedure (4), when the first approach to conservative treatment is not satisfying is related to corticosteroid injections (5) adjacent to the area of maximum swelling.

Pulsed Nd-YAG laser therapy demonstrated to be a beneficial tool for the conservative treatment of insertion syndrome, in combination with manual therapy and NSAIDs assumption to manage pain peak. The possible mechanism for the action of pulsed Nd-YAG laser therapy in intersection syndrome is related to its anti-inflammatory properties (11), which have been documented in scientific studies along with its anti-oedemigenic and analgesic effects (12, 13).

High intensity laser therapy thanks to its anti-inflammatory properties can be a beneficial tool in the conservative management of intersection syndrome in athletes. The present case suggests that the appropriate treatment approach, integrating traditional and inno-

vative tools, allows for a full recovery with no relapses, even when sport activity is started again extensively.

Conflict of interest: None to declare

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Case report

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia in a young man with hypoxia: a case report and review of the literature

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Summary. Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is a rare lung disease, which usually affects older women. This disease is often asymptomatic. For patients who are symptomatic, symptoms usually include cough and dyspnea. In this paper, we reported a 38-year-old man who suffered from chest pain for 3 months. CT scan findings revealed scattered nodules that were less than 1 cm. Spirometry was normal and the arterial oxygen saturation at room air was 85%. Open lung biopsy revealed DIPNECH. Patients with DIPNECH are mainly elderly women with symptoms including cough and dyspnea. However, we reported a young man with chest pain and hypoxia without dyspnea. DIPNECH can occur in male and female individuals at any age. (www.actabiomedica.it)

Key words: diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

Introduction

Neuroendocrine tumors originate from neuroendocrine cells. Due to the scattering of these cells in the digestive and respiratory tracts, these two organs are the most common sites of such tumors. When neuroendocrine cell hyperplasia is idiopathic and limited to the epithelium, it is called diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH). If this hyperplasia passes through the basement membrane and is less than 5mm in diameter, it is called Tumorlet, but if the diameter is more than 5mm, it is called carcinoid. (1) DIPNECH and Tumorlet can act as carcinoid precursors (1). DIPNECH was first described by Aguayo in 1992 (2). In carcinoids, there is a disturbance in the 11q3 region while it is not seen in Tumorlet (3). Therefore, this genetic mutation may be the cause of carcinoids. Most cases of Tumorlet and DIPNECH are presented in combination with other lung diseases like fibrosis and bronchiectasis (4). Hence, this cell proliferation is likely to be a tissue reaction.

Although carcinoid and neuroendocrine tumors account for about 1% to 2% of lung tumors (1,5), the incidence of DIPNECH and Tumorlet is unpredictable. Specific clinical symptoms have not yet been defined for Tumorlet and DIPNECH. However, most of the reported patients presented only symptoms including cough and shortness of breath. Most of the reported cases were middle age women who were non-smokers. In this study, we introduced a 38-year-old man who had hypoxia due to Tumorlet.

Case presentation

The patient was a 38-year-old non-smoker man without any history of diseases. He was admitted at a clinic with chest pain without dyspnea, cough, hemoptysis or constitutional symptoms. His vital signs were stable and there were bilateral inspiratory crackles in his both lungs. Arterial oxygen saturation was 85% at room air and the rest of the examination was normal.

Chest X-ray revealed diffuse bilateral nodular opacity. CT scan showed random bilateral diffuse nodules of about 3-9mm diameter (Figure 1). Spirometry was normal (FEV1/FVC = 83%, FEV1 = 3.6 L (78%), FVC = 4.3 L (81%). Open lung biopsy from RLL was carried out. In lung biopsy, small nodules of uniform cells were seen in the form of cell nests. These cells contained oval to spindle shaped nuclei, granular chromatin and eosinophilic cytoplasm (Figure 2). These cells in IHC were positive for NSE and chromogranin but negative for vimentin and actin. The cellular mitotic activity index was less than 7%; considering the morphology, positive neuroendocrine markers and low mitotic index, DIPNECH was diagnosed.

Discussion

Although about 90% of patients with Tumorlet and DIPNECH are old women, (6), our patient was a young man. Symptomatic patients usually have dyspnea and chronic cough. However, the patient in our study had only chest pain; he was hypoxic without any spirometric impairment.

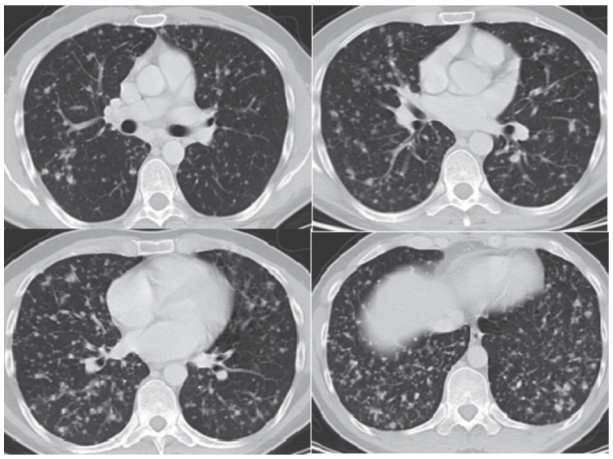

Figure 1. CT scan presents diffuse nodular opacity

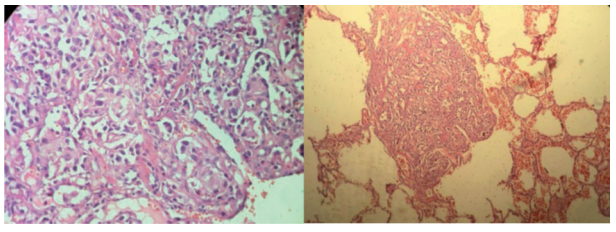

Figure 2. The nodules of the neuroendocrine cell nest pattern. The cells contain oval shaped nucleus and granular chromatin

Nearly 200 cases of DIPNECH have been introduced. DIPNECH may be detected accidentally in CT scan or pathology in patients who are evaluated for other reasons (6). If the patient is symptomatic, spirometry usually exhibits obstructive or restrictive patterns (7). In CT scan, the presence of scattered nodules in the lung, especially when accompanied by air trapping, is considered to be the typical finding of DIPNECH. (8) Tumorlet has been highly-absorbed in PET scan (9,10). Diagnosis of DIPNECH is usually based on open lung biopsy. Since the lesion originates from the bronchial epithelium, trans-bronchial lung biopsy or cryobiopsy can also be useful (9,11).

Marcheusky and colleagues proposed the criteria for diagnosis of DIPNECH by examining 70 pathology samples of surgical patients who had neuroendocrine cell hyperplasia (12). They found that patients who had at least three areas of Tumorlet in addition to multi-focal neuroendocrine hyperplasia were exposed to carcinoid, and that this criterion is necessary for the diagnosis of DIPNECH. However, since many patients are asymptomatic, this definition cannot be applied clinically. That is why Giulio and colleagues used the term DIPNECH syndrome or "DIPNECH with airway disease" (6). This term is used for those with a long term history of chronic pulmonary symptoms with an obstructive pattern in spirometry, abnormal findings in CT scans and neuroendocrine hyperplasia in pathology. At such circumstances, treatments should be focused on constrictive bronchiolitis

The course of the disease is different. Most patients are stable. However, some of them may develop advanced pulmonary obstructive diseases due to constrictive bronchiolitis, which can even lead to lung transplantation. If the patient is asymptomatic and has no progressive symptoms, no treatment is required. Tumorlet may transform to carcinoid and thus follow-up of the patient based on biopsy, clinical findings, spirometry, and imaging is recommended (13-15).

Based on the data in the literature and according to Marchevsky's pathological definition and Giulio's classification, we designed an algorithm to approach neuroendocrine hyperplasia (NECH) observed in the

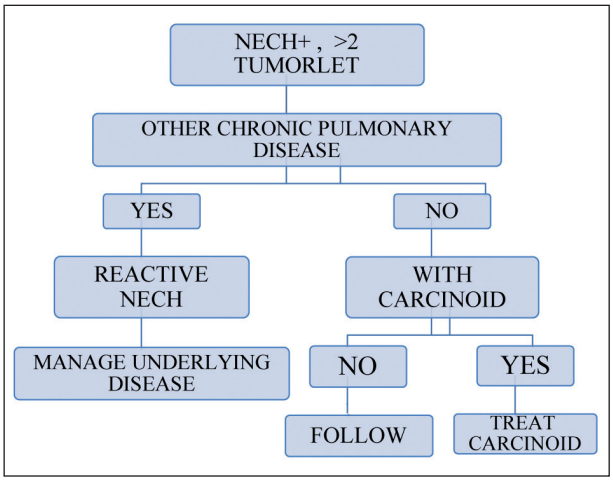

Figure 3. Flow chart to manage DIPNECH. NECH: neuroendocrine cell hyperplasia

pathology (Figure 3). This diagram provides a good concept for understanding of DIPNECH.

The uniqueness of our case was that, despite very low prevalence of the disease in men and at early ages, our patient was a young man. Moreover, the patient had hypoxia without a spirometric abnormality. DIPNECH seems to be associated not only with an obstructive mechanism but also with diffusion problems, leading to respiratory disorders. In our patient, chemotherapy was initiated due to hypoxia. However, after one year, there was no change in PFT and CT scans. Currently, the patient's functional class is suitable and he is not receiving any treatments.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

Isolated fronto-ethmoidal allergic fungal rhinosinusitis: case report and review of the literature

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Summary. *Background and aim of the work:* Fungal rhinosinusitis (FRS) is a clinical entity characterized by the presence of fungi within sino-nasal cavities that may occur in patients with normal or defective immunity. Allergic fungal rhinosinusitis (AFRS) is a form of non-invasive FRS that affects patients with an abnormal immuno-mediated response to fungal antigens. This article describes a case of isolated fronto-ethmoidal AFRS. *Methods:* A 20-year old male patient presented with a history of a left nasal respiratory obstruction and allergic oculorhinitis. CT scans showed a polypoid mass in the left nasal cavity and opacification of the left ethmoid sinus, frontal recess and frontal sinus with hyperdense component. The patient underwent functional endonasal sinus surgery (FESS) with removal of nasal polyps from the left nasal cavity and of cheesy-like material and dense mucus from the left ethmoid and frontal sinus. Histological examination showed presence of fungal hyphae within the allergic mucus; a diagnosis of AFRS was made. *Results:* Follow up at 14 months showed no signs of recurrence. *Conclusions:* The AFRS case reported herein is characterized by isolated unilateral fronto-ethmoid involvement, a rare presentation. Endoscopic nasal treatment was effective with complete patient recovery. (www.actabiomedica.it)

Key words: ethmoid, frontal, fungal, rhinosinusitis, allergic, sinus

Introduction

Chronic rhinosinusitis (CRS) is a common inflammatory condition of the mucosa of the nasal and paranasal cavities affecting even up to 13% of the population (1). Fungal rhinosinusitis (FRS) is part of the spectrum of CRS. Over the years, mycotic infections of the nose and paranasal sinuses have been more clearly defined, although some features concerning its physiopathology, diagnosis and management remain challenging.

Sinus mycosis can occur in individuals with normal immune system as well in patients with immunodeficiency. Deficiency of protective mechanisms, such as mucosal properties, cellular and humoral immunity, and bacterial antagonism are central for FRS development (1).

Allergic fungal rhinosinusitis (AFRS) is a non-invasive form of FRS with a prevalence of 6-9% (2). Typically, AFRS is unilateral and involves multiple paranasal sinuses. The ethmoid sinus is the most frequent localization and it's rarely involved alone; the frontal sinus is very rarely affected (3). We describe a rare case of AFRS in a young man involving only the ethmoid and frontal sinuses.

Case report

We report a case of a 20 year-old male patient with a history of left nasal respiratory obstruction and allergic oculorhinitis. He had positive Prick tests for *Dermatophagoides spp., graminacee, Parietaria, Alter-*

naria (molds) and *cat epitelium*. Pre-operative evaluation showed signs of rhinitis and left deviation of nasal septum. The eosinophils blood count was normal.

Pre-operative CT scan showed a polypoid mass in left nasal cavity, opacification of the left ethmoid sinus with a hyperdense component, obliteration of the left ostio-meatal complex, opacification of the left frontal recess and of the correspondent sinus and a right concha bullosa (figure 1). A diagnosis of chronic rhinosinusitis with fungal infection was suggested . Surgical treatment was proposed to the patient.

The patient underwent functional endonasal sinus surgery (FESS), frontal and ethmoid sinus mucus removal and Draf IIA on the left side. Intra-operatively dense mucus of the frontal sinus and cheesy-like material from the ethmoid sinus were removed and sent for histopathological examination (figure 2). Maxillary sinus was found empty with no pathological findings.

Histological examination showed fungal septate dichotome hyphae consistent with species of *Aspergillus*, allergic mucus and polypoid inflammatory hyperplasia of the sino-nasal mucosa (figure 3). These findings are consistent with a diagnosis of AFRS. Follow up at 14 months showed no signs of recurrence.

Discussion

Fungal spores, due to their ubiquitous nature, are continuously inhaled and deposited in the airway mucosa. Fungi are normal pathobionts of the mammalian respiratory tract that, under conditions of abnormal immune response, may cause overt disease. CRS can progress from a local to a systemic allergic reaction; the prevalence of AFRS and FRS is 1,3% and 2,8% respectively in CRS patients (2). Fungal sinusitis results from interaction of different factors, such as the integrity of upper airways mucosa, the expression of antimicrobial proteins on the ciliated epithelium and the host's immune response (4). A protective role of Th17 cells and IL-17A in defense against fungal infections has been implicated (5). Environmental conditions, such as high air concentration of fungal spores at certain workplaces (farmers, gardeners, carpenters)

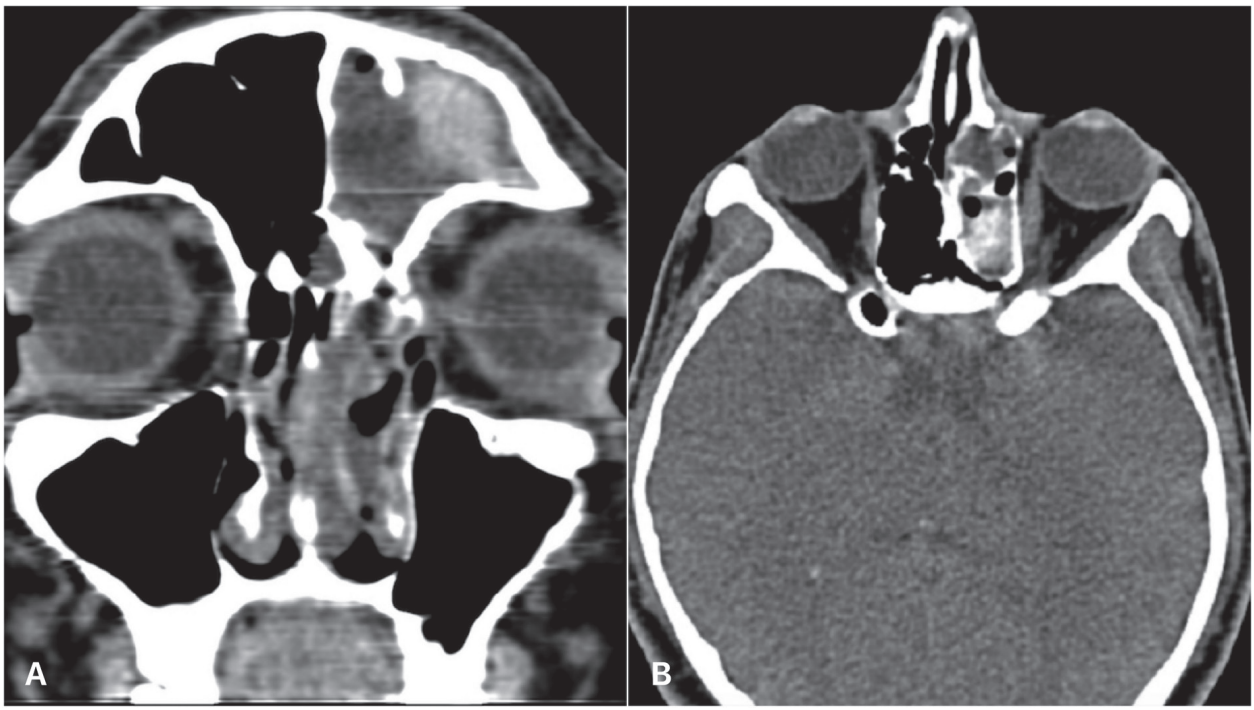

Figure 1. Pre-operative CT scan shows left nasal polips and opacification of the left frontal and ethmoid sinus (a, coronal image) with hyperdense component in the ethmoid sinus (b, axial image)

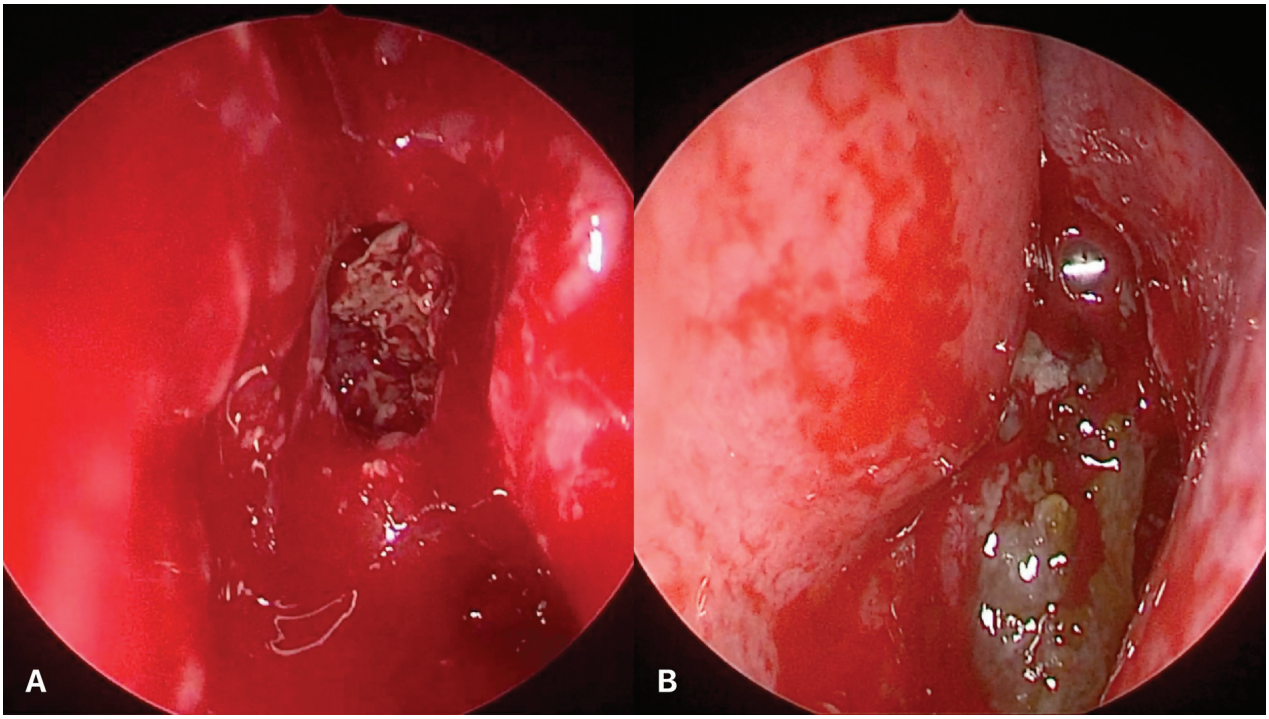

Figure 2. Endoscopic findings: inflammatory mucus within the frontal sinus (a); allergic mucus within the ethmoid sinus (b)

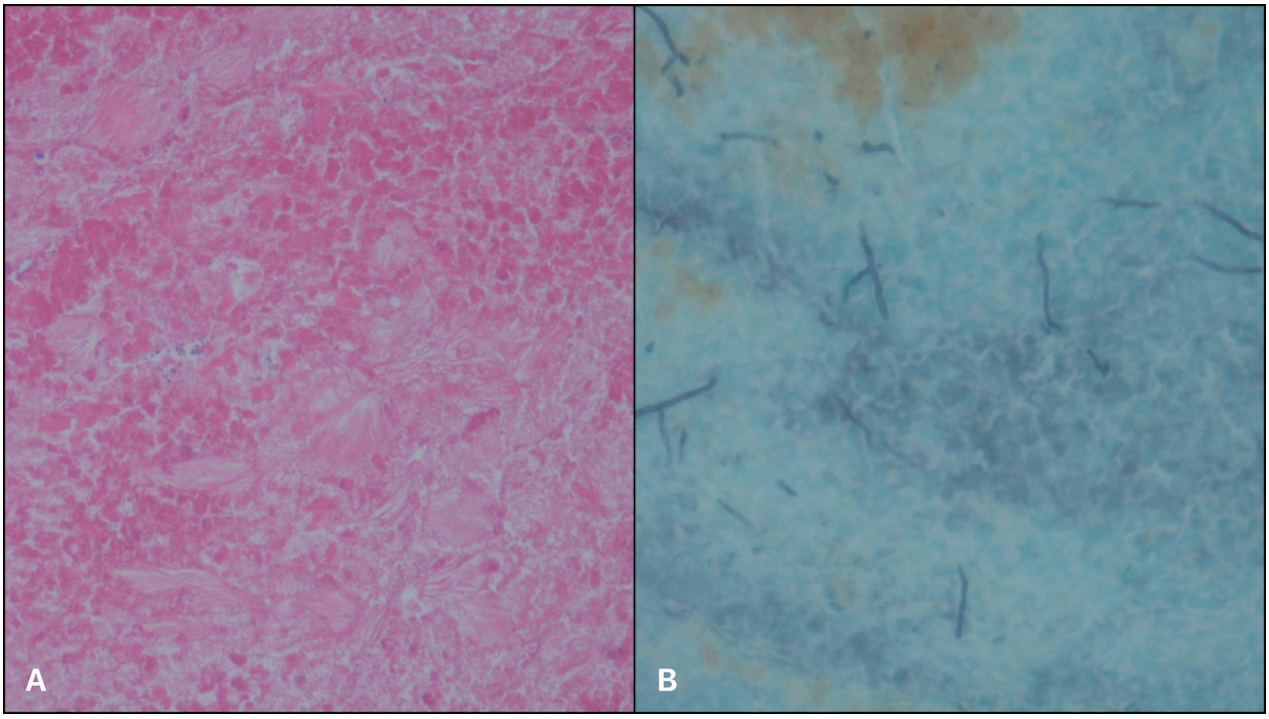

Figure 3. Histologic examination showed necro-inflammatory material (a, Haematoxylin-eosin staining; original magnification, x20) with fungal hyphae (b, Grocott's metamyne silver stain; original magnification, x40).

Table 1. Previous studies on CT findings in AFRS (PE=posterior ethmoid; AE=anterior ethmoid; NR=not reported; E=ethmoid)

Author, year	Patients number (age range)	Ethmoid sinus involvement (%)	Maxillary sinus involvement (%)	Sphenoid sinus involvement (%)	Frontal sinus involvement (%)	Isolated involvement of ethmoid, frontal sinus or both	*Isolated species*
Samir et al, 1996	14 (9-50 y)	11 (79%)	9 (64%)	8 (57%)	6 (43%)	0	*Aspergillus fumigatus 57%* *Aspergillus flavus 14%*
Torres et al, 1996	16 (8-71 y)	16 (100%)	13 (81%)	13 (81%)	5 (31%)	0	*Exserohilum 19%* *Bipolaris sp. 6%* *Curvularia 6%* *Drechslera 6%*
Mannign et al, 1997	10 (13-57 y)	9 (90%)	4 (40%)	6 (60%)	5 (50%)	1 isolated PE involvement (10%)	*Bipolaris sp. 40%* *Exserohilum sp. 20%* *Curvularia sp. 10%* *No growrth 20%*
Mukherji et al, 1998	45 (8-68 y)	43 (96%)	42 (93%)	30 (67%)	32 (71%)	1 isolated E involvement (2%)	*NR*
Nussembaum et al, 2001	28* (NR)	24 (86%)	2 (7%)	22 (79%)	8 (29%)	NR	*Bipolaris sp. 57%* *Curvularia 14%* *No growth 18%* *Others 11%*
Aribandi et al, 2007	7 (8-67 y)	6 PE (86%) 5 AE (71%)	3 (43%)	6 (86%)	4 (57%)	NR	*Bipolaris sp. 100%*
Wise et al, 2009	111	77%	68%	58%	53%	NR	*NR*
Verma et al, 2016	100	AE 92 (92%) PE 84 (84%)	76 (76%)	67 (67%)	65 (65%)	NR	*Aspergillus flavus 64%*

or in humid and poorly ventilated rooms may also predispose to fungal sinusitis (6-9). Climatic factors, such as temperature and humidity, may explain differences in the prevalence of mycoses in different geographic areas.

There are five forms of fungal disease that affect the nose and the paranasal sinuses: acute invasive FRS (including rhinocerebral mucormycosis), chronic invasive RHA, invasive granulomatous FRS, mycetoma (fungus ball) and non-invasive AFRS (4).

AFRS is a disease of young adults, with an average age of 26 years at time of diagnosis (10). It is often associated with nasal polyps (NP), IgE-mediated hypersensitivity, and allergic mucin (2, 11). Since the early 90's, several studies have described the CT findings in AFRS that are characterized by multiple sinus involvement or pansinusitis in the majority of the cases. Ethmoid sinus is the most frequently involved site, followed by the maxillary, sphenoid and frontal sinuses. Although AFRS is a predominately unilateral disease, isolated involvement of the frontal and ethmoid sinus represents a rare finding in literature.

In 1996, Samir and Torres (12, 13) described the CT findings in 30 AFRS cases; none of them had isolated involvement of frontal and ethmoid sinus. In 1997, Manning et al. (14) reported 10 cases of AFRS, including one case of isolated ethmoid involvement. A series of 45 cases was reported by Mukherji et al. (15) with only one case of isolated ethmoid involvement. Gupta et al. (3) reported 12 cases of isolated AFRS of the frontal sinus. Other authors (16-19) reported frequencies of involvement of the ethmoid (71-92%), maxillary (7-76%), sphenoid (58-86%) and frontal sinus (29-65%) in AFRS, but no cases of isolated fronto-ethmoid involvement were described. Different species have been identified by cultures; the most frequent are *Aspergillus* species followed by *Bipolaris, Exserohilum* and *Curvularia* species. The previous studies are summarized in Table 1.

The AFRS case reported herein is characterized by isolated unilateral fronto-ethmoid involvement, a rare presentation. Endoscopic nasal treatment was effective with total removal of allergic mucin and inflammatory material within the sinuses and complete patient recovery.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

De Garengeot hernia: laparoscopic treatment in emergency

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Summary. *Introduction:* De Garengeot Hernia is described as the presence of an appendix within a femoral hernia. *Case Report:* We report the case of an elderly woman, who presented with incarcerated femoral hernia without signs of bowel obstruction. CT showed a femoral hernia with appendix in the femoral canal with signs of strangulation. the patient underwent emergency surgery. Diagnostic laparoscopy revelead a non-reducible appendix in the femoral canal, in the absence of signs of peritonitis. An infrainguinal incision was performed. An gangrenous appendix within the sac was revealed, detached from the sac and reintroduced into the abdomen through the femoral canal. The laparoscopic appendectomy was then performed. The hernia repair was performed by suturing the iliopubic tract to Cooper's ligament. Patient had a regular course. *Discussion:* De Garengeot's hernia is a rare occurrence. After the year 2000 a total of 32articles, wich presented 34 cases of de Garengeot's hernia have been published. Due to the rarity of this disease there is not standard procedure; laparoscopy may be a valid technique for determining the condition of the hernia, but due to the difficulty of preoperative diagnosis it is unlikely to be the first choice for the surgical approach. The use of CT can therefore be decisive to help the surgeon in the choice of the approach. *Conclusion:* De Garengeot's hernia can be approached in urgent laparoscopy even in the complicated forms of appendicular inflammation. (www.actabiomedica.it)

Key words: De Garengeot, femoral hernia, appendicitis, laparoscopy

Introduction

Femoral hernia consists in a projection of the sac through the femoral triangle, below the inguinal ligament and presents with incarceration in about 50% of cases. The femoral hernias account for less than 3% of all hernias, have a rate of incarceration ranging between 5 and 20% and their content in typically omentum (1).

De Garengeot Hernia is described as the presence of an appendix within a femoral hernia, usually discovered inidentally during femoral hernia repair. This phenomenon occurs in 0,5-5% of all femoral hernias, while the presence of an appendicitis within a femoral hernia is even more rare, occurring in 0,08% to 0,13% of all cases (2).

This rare condicion was first described in 1731 by the french surgeon Rene Jacques Croissant de Garengeot (3).

But only in 1975 Hervin performed the first appendectomy in an incarcerated femoral hernia (4). To date there have been approximately less than 100 cases reported in the literature (5).

Case Report

MO, a 82-year-old female was admitted in our hospital with an irreducible swelling in the right groin region associated with localized pain and fever about 10 days. There were no abdominal pain, nusea, vomiting.

The medical history of patient reported hypertension and no previous abdominal surgery. The abdominal medical examination revealed only a painful and irreducible lump in the right inguino-crural region with overlyning skin erythema. Her abdomen was soft, non-distended with normal bowel sounds on auscultation and no signs of bowel obstruction.

A small increase of infiammatory markers was noted un her blood tests (WBC=13,270 x10^9/L, CRP =11,98/dl).

The patients pulse rate was 110/min, blood pressure of 110/70 mmHg, respiratory rate of 15/min, body temperature of 37,2°C, and oxygen saturation of 98.% on room air.

The abdominal X ray did not show signs of bowel obstruction.

The CT scan showed a femoral hernia with appendix in the femoral canal anteromedial to the femoral vessels with signs of strangulation, air bubbles and fluid in the hernia sac (Figs. 1, 2, 3, 4); the patient underwent emergency surgery.

The decision was taken to perform laparoscopic surgery with general anaesthesia. An infraumbilical incision was made, and a pneumoperitoneum was obtained using Hasson's open technique. Two 5-mm abdominal port were placed suprapubically and in right lumbar quadrant. A 12-mm port in the left iliac fossa. A 10-mm, 30-degree telescope was used to examine the abdominal cavity. Diagnostic laparoscopy revelead a non-reducible appendendix in the femoral canal, in the absence of signs of peritonitis (Fig. 5). An infrainguinal incision was performed. The femoral hernia was found out and was opened. An gangrenous appendix within the sac was revealed, detached from the sac and reintroduced into the abdomen through the femoral canal. The laparoscopic appendectomy was then performed with electrodissection of the mesenteriolum, endoloop placement at the base and extraction of the appendix in

Figure 1.

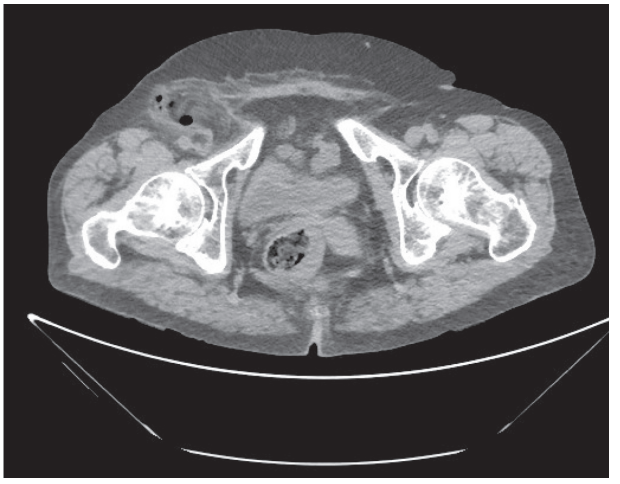

Figure 2.

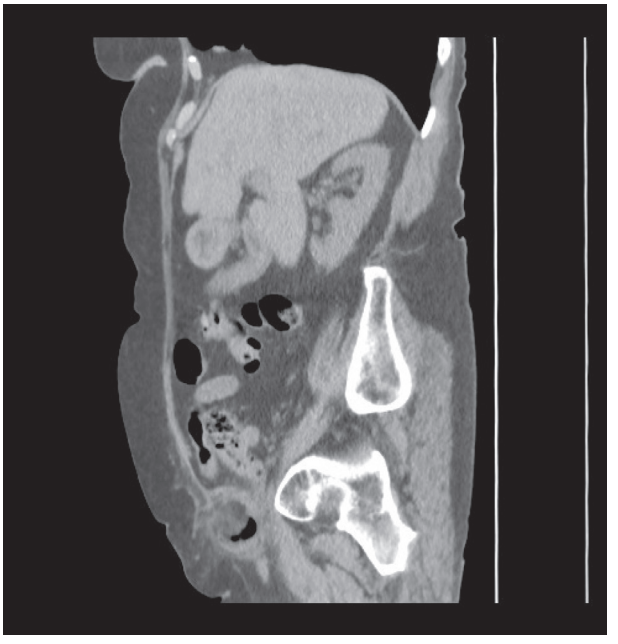

Figure 3.

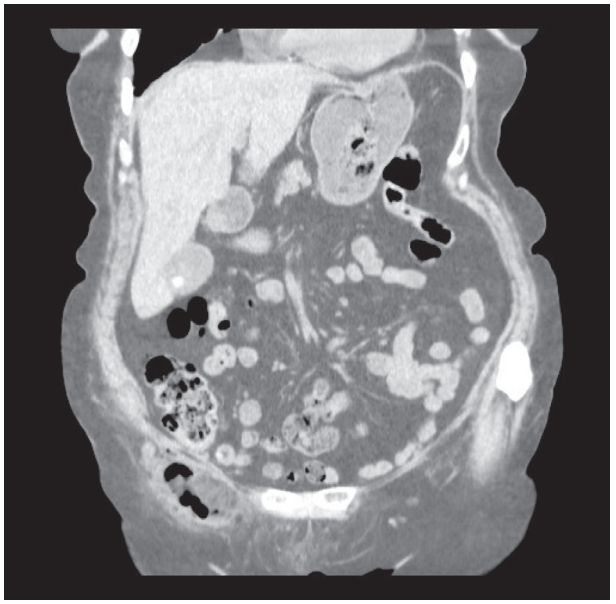

Figure 4.

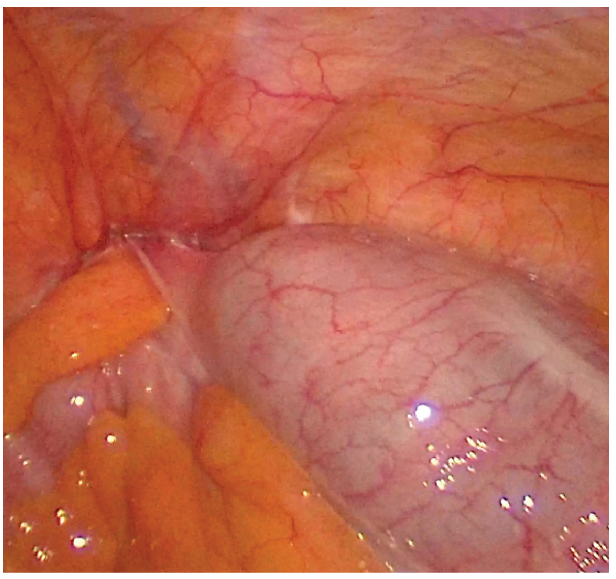

Figure 5.

endobag through the trocar. a drainage tube was placed through the right trocar, then removed on the fifth day.

The hernia repair was performed by suturing the iliopubic tract to Cooper's ligament with non-absorbable suture materials after sac removal, without use of a mesh. The histological examination confirmed a gangrenous appendicitis.

Patient had a regular course and was discharged on the eighth postoperative day.

Discussion

De Garengeot's hernia is a rare occurrence. The inflammatory process of the appendix is usually caused by the extraluminal obstruction of the appendix at the hernial neck, rather than by the more usual cause of intraluminal obstruction (6). The obstruction of the neck leads to a vascular compromise which allows bacterial overgrowth (7). The anatomy of the femoral canal usually prevents intraperitoneal spread of infection and the patient does not present signs of peritonitis (8).

After the year 2000 a total of 32 articles, wich presented 34 cases of de Garengeot's hernia and histology proven appendicitis have been published. Several surgical approaches have been described. Most surgical strategies began with an inguinal incision with appendectomy performed through the hernial defect and subsequent repair of the hernia with or without mesh (9-11). Six patiens had a laparotomy with a lower midline incision, because of a high possibility of abscess or perforation (9). Only in 2 published cases a laparoscopic approach was performed: in both cases, unlike the case that we present in this report, the appendix was reducible in the abdomen during the laparoscopic surgery and proceeded both to the appendectomy and to the repair of the hernial defect in laparoscopy, in a case with mesh (TAPP) and in a case with direct repair (12, 13).

Due to the rarity of this disease there is not standard procedure (13); laparoscopy may be a valid technique for determining the condition of the hernia, but due to the difficulty of preoperative diagnosis it is unlikely to be the first choice for the surgical approach (14).

The use of CT, not always practiced in published cases, for the early diagnosis in the preoperative phase of this type of hernia, can therefore be decisive to help the surgeon in the choice of the approach (15).

The use of the mesh is controversial: for many authors it should be reserved for cases in which there is no appendix inflammation and risk of infection (16) although some studies prove the feasibility of implantation of mesh even in infected wounds (12, 17).

Conclusion

De Garengeot's hernia can be approached in urgent laparoscopy even in the complicated forms of appendicular inflammation, especially when a preliminary CT study leads to correct diagnosis. The execution of the appendectomy by laparoscopy, can be advantageous going to avoid the execution of a median laparotomy in cases where it is not possible to safely perform the appendectomy through the inguinal incision.

Consent: Informed consent wes obtained from the patient for publication and avaible upon request.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

Case report of atypical endometrial stromal cells in an endometrial polyp and osteoclastic like giant cells in leiomyoma in the same patient: Is it a coincidence or is it a result of Tamoxifen treatment?

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Summary. Atypical stromal cells are rarely identified in the endometrial polyps of the female lower genital system. These cells are suggested to develop due to a degenerative or reactive phenomenon. And osteoclastic like giant cells, which have been reported in many epithelial and mesenchymal tumors of uterus, may develop because of a degenerative or a reactive process. In this case report, we present a breast cancer patient who was commenced on Tamoxifen treatment presenting with formation of both atypical stromal cells in an endometrial polyp and osteoclastic like giant cells in the leiomyoma of the uterus. Patients who are treated with Tamoxifen should be followed meticulously to detect tumoral or proliferative lesions of endometrium and myometrium because of the adverse effects of tamoxifen on uterus. (www.actabiomedica.it)

Key words: atypical endometrial stromal cells, osteoclastic like giant cells, leiomyoma, tamoxifen treatment

Introduction

Fibroepithelial polyps of the female lower genital system are periodically observed while atypical stromal cells are very rarely reported as a feature of the endometrial polyps (1). Atypical stromal cells are described for the first time in an endometrial hyperplastic polyp in 1995 by Creagh et al (2). These cells are stellate and enlarged, display moderately to severely atypical, hyperchromatic, multilobulated nuclei, and have little proliferative activity with no mitoses (3). Atypical stromal cells inside the endometrial polyps are suggested to develop due to a degenerative or reactive phenomenon (1).

Tamoxifen which has antiestrogenic properties, exerts a weak estrogenic effect on human endometrium leading to a spectrum of endometrial proliferative lesions like polyps, simple/complex atypical hyperplasia, and even adenosarcomas, depending on the patients' menopausal status; as well as on the dose and duration of tamoxifen usage (4).

Osteoclastic like giant cells have been reported in various epithelial and mesenchymal tumors in various anatomic locations including uterus ,5. Except for the malignant tumors in literature; there is one case revealing osteoclastic like giant cells in uterine leiomyoma (5).

In this case report, we present a breast cancer patient who was commenced on tamoxifen treatment that led to the formation of both atypical stromal cells in an endometrial polyp and an osteoclastic like giant cells in the leiomyoma of the patient.

Case report

Our patient is a 53 years old woman, who is Caucasian in origin. In 2013, radical mastectomy procedure

had been performed for invasive ductal carcinoma of breast. After surgery, 10 cycles of chemotherapy were given to the patient. Following chemotherapy, she used Tamoxifen between 2014 and 2017. The dose of Tamoxifen was 20 mg/day. She had no any other disease in her general examination. Before using Tamoxifen, transvaginal ultrasonography had been performed, and everything was normal in her ultrasonography. She did not have any complaint in her follow-ups. She was admitted to the gynecology and obstetrics clinic for pelvic pain in November 2016. After her physical examination and transvaginal ultrasonography examination and her endometrium was found thickened in ultranography. Therapeutic curettage was performed due to this increase in endometrial thickness. In histopathological examination of this curettage specimen, significant stromal atypical cells were detected in the superficial endometrial stroma. These atypical cells were stellate, spindle and enlarged cells. Their nuclei were hyperchromatic and had no mitoses. The cells revealed dense chromatin with prominent nucleoli (Figure 1, 2). Immunohistochemically CD10 was positive in the atypical stromal cells while H-caldesmon and pan cytokeratin were negative in these cells. However, these atypical stromal cells in the curettage specimen were so scarce that these cell groups were not apparent in the proceeding sections of the specimen. With immunohistochemical and morphological findings the curettage specimen was reported as suspicious for malignancy with the differential diagnosis of adenosarcoma and high-grade stromal sarcoma. Total abdominal hysterectomy and bilateral salpingo-oophorectomy was performed. In the macroscopic examination of the patient's uterus: a 5×2,5×1,5 cm grey-brown colored polypoid lesion was detected in the endometrial cavity. In the hysterectomy specimen of the patient there were three intramural white, fasciculate nodules, measuring between 1 and 6 cm in diameter. These intramural nodules did not have any necrotic or hemorrhagic areas. Any other significant atypia was found in the endometrial polyp. The findings were compatible with classical endometrial polyps (Figure 3).

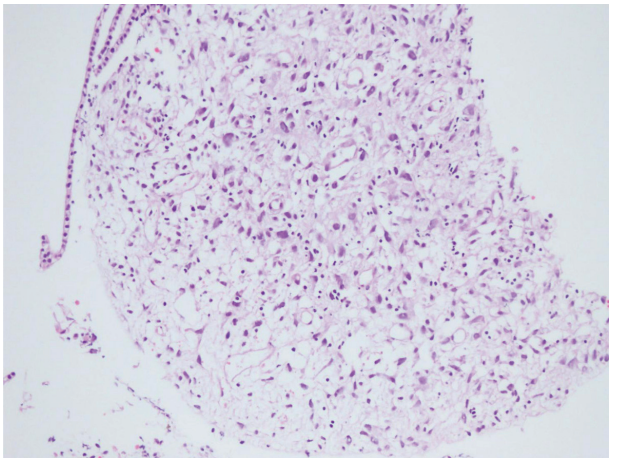

Figure 2. Pleomorphic, enlarged stellate cells with hyperchromatic nucleus (HE X 200)

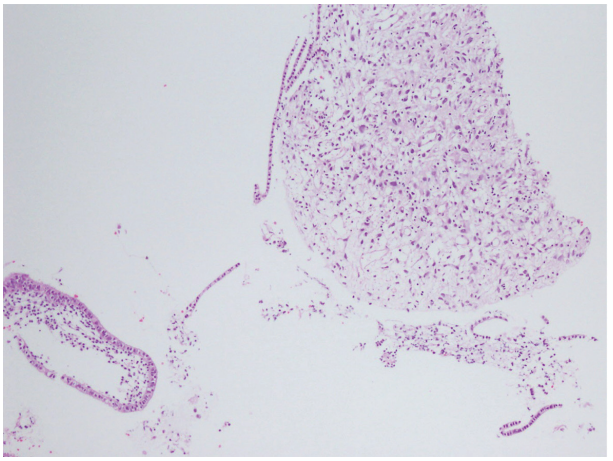

Figure 1. Small atypical stromal cell group in curettage specimen (HE X 100)

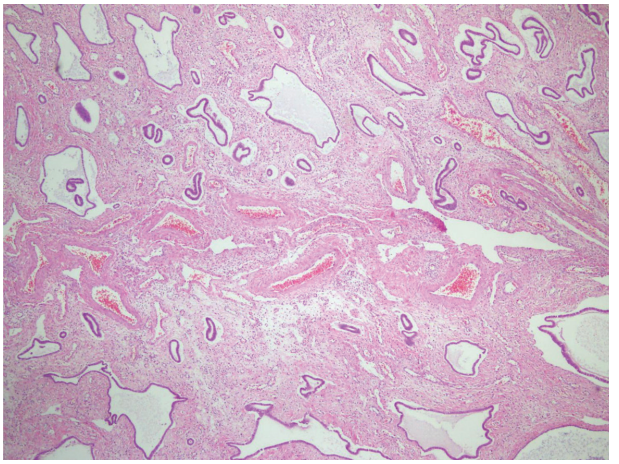

Figure 3. Classic appearance of endometrial polyp in resection (HEx40)

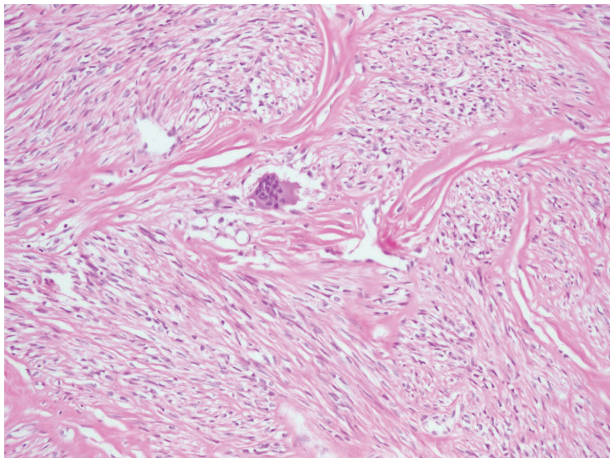

Figure 4. Osteoclastic like giant cell in the leiomyoma section (HE X 200)

The cervix, fallopian tubes and ovaries were ordinary. One of the leiomyomata, measuring 3.7 cm, had infrequent benign-appearing osteoclastic like giant cells between the smooth muscle fibers. Osteoclastic like giant cells were diagnosed morphologically (Figure 4). The uterus specimen was histopathological reported as an endometrial polyp and leiomyoma with osteoclastic like giant cells. After the operation, the patient had been examined with transabdominal ultrasonography again. So far, she had no other complaint.

Discussion

Fibroepithelial polyps of female the lower genital system is frequently observed, and atypical stromal cells are rarely present as a feature of the endometrial polyps in patients ranging from 24 years to 86 years (1). However, these cells have been rarely described in proliferative endometrium without polyps (6). Atypical stromal cells of the lower genital tract were first described in vagina by Norris and Taylor (2). Later these cells were reported in the vulva and cervix (7). In 1992 a case of bilateral fibroepithelial polyp of labia minora containing atypical stromal cells was published (2). Creagh et al, described this phenomenon for the first time in 1995 in an endometrial hyperplastic polyp (2). Tai et al, reported 15 cases with an average age of 59.7 years in 2002 (2). In this report, 12 patients were in post-menopausal period and five patients had hormone replacement therapy history (2).

Atypical stromal cells are stellate and enlarged cells (3). Their nuclei are moderately to severely atypical, hyperchromatic, multilobulated, and show little proliferative activity with no mitoses (3). They have dense chromatin and prominent nucleoli (3). Pseudo inclusions may be present (2, 3). Cells are invariably positive for vimentin, estrogen, androgen, and progesterone receptors occasionally and focally for CD10 which is a frequent endometrial stromal marker for smooth muscle markers such as actin, desmin, h-caldesmon and occasionally for S100 which is a Schwann cell and lipid cell marker (3). On the other hand, atypical stromal cells are consistently negative for cytokeratin, epithelial membrane antigen, myogenin, CD34, antchymotrypsin, Factor VIII, and lysozyme (3).

The origin of atypical stromal cells is controversial (2). They are suggested to originate from fibroblasts and their differential potential into both endometrial stromal and smooth muscle cells has been documented (8). It is thought that these cells consist of a primitive cellular population originating from the multipotent mesenchymal cells ,8. Atypical stromal cells inside the endometrial polyps develop due to a degenerative or reactive phenomenon like in atypical (bizarre) leiomyoma (1). ln order to explain the pathogenesis of these cells, several hypotheses have been proposed. One of them is that the mast cells may play a role in the formation of multinucleated stromal cells because these cells are essential for the development of inflammation, fibrosis, and angiogenesis (9). In addition to these hypotheses, because of the electron microscopical findings and reaction with S100 antibody, atypical stromal cells are thought to originate from peripheral nerve sheath (10). Besides these hypothesis, atypical stromal cells may also have either a smooth muscle or an endometrial stromal immunophenotype (6).

Because the curettage material with limited specimen volume, differential diagnosis of atypical stromal cells from adenosarcoma, endometrial stromal sarcoma, and carcinosarcoma/malignant mixed Mullerian tumor (MMMT) is crucial and must be meticulously done (8). Atypical stromal cells are distinguished from adenosarcoma by the lack of stromal hypercellularity, peri glandular stromal cuffing, cambium layer beneath

the surface epithelium, leaf like projections into the glandular Lumina, increased stromal mitotic activity which is >3 mitosis/10HPF and sarcomatous stromal overgrowth with homologous and/or heterologous elements (2). Differentiation from endometrial stromal sarcoma is by endometrial stromal sarcomas appearance as a mass lesion with infiltrative borders (2). Endometrial stromal sarcomas are tan to yellow and can be bright orange in color. Microscopically they are composed of tightly packed spindled cells without significant glandular component (2). There are small capillaries distributed evenly in these lesions (2). Occasionally, starburst hyalinization is noted (3). Endometrial stromal sarcomas of uterus present as worm like masses throughout the uterus and typically do not form pure polypoid lesions, although they may have a polypoid component (8). Atypical cells are extremely rare in endometrial stromal sarcomas (8). Malignant mixed Mullerian tumor is a rare neoplasm, also termed uterine carcinosarcoma comprising only 1-2% of uterine neoplasms (11). These tumors are a dedifferentiated or metaplastic form of endometrial carcinoma (12). This tumor reveals both glandular and stromal components (2). Sarcomatous component may contain homogenous and/or heterogenous elements (2). The characteristic microscopic feature is an intimate admixture of malignant epithelial and stromal elements (11). Although increased mitotic activity among the atypical cells is supportive for carcinosarcoma in a curettage or biopsy sample, the possibility of MMMT is difficult to exclude because of the fragmented nature of the specimen and the possibility of sampling error (2).

Tamoxifen is a nonsteroidal triphenylmethyl compound that is used as adjuvant therapy in the treatment of breast cancer patients (4). It is a selective estrogen receptor modulator (SERM) and prolongs overall and disease-free survival in breast cancer (13). It reduces the likelihood of disease development in the contralateral breast and also may reduce the risk of development of breast cancer in asymptomatic women with a strong family history (13). Tamoxifen affects by antiestrogenic properties that are mediated by competitive binding to the estrogen receptor (4). However, it also has a weak estrogenic effect on human endometrium (13). The endometrium of postmenopausal patient who use tamoxifen is significantly thicker than that of age matched group who do not use tamoxifen (14). Ki-67 proliferation index is higher in such patients (14). Tamoxifen results in a spectrum of endometrial proliferative lesions like polyps, simple/complex atypical hyperplasia, and even adenosarcomas (4, 14). McCluggage et al, reported that high grade endometrial cancers including serous carcinomas and carcinosarcomas are more frequent in patients using tamoxifen (15). They also revealed that uterine leiomyosarcomas, endometrial stromal sarcomas, adenofibromas, and adenosarcomas may occur in patients using tamoxifen (15).

Osteoclastic like giant cells have been reported in numerous epithelial and mesenchymal tumors in various anatomic locations mostly in uterus. They are present in endometrial carcinoma, endometrial Aden squamous carcinoma, endometrial stromal sarcoma, extraosseous malignant giant cell tumor and malignant fibrous histiocytoma and leiomyosarcoma (16, 17). Except for the malignant tumors in the literature; there is one case revealing osteoclastic like giant cells in uterine leiomyoma (5). In this case Guilbert et al, have described benign appearing osteoclastic like giant cells in a leiomyoma, measuring was 3,5 cm. These cells were admixed with smooth muscle bundles (5). Bizarre multinucleated giant cells have been reported in atypical leiomyomas (5). But in contrast to osteoclastic like giant cells's monotonous morphological features, multinucleated giant cells in bizarre leiomyoma show varying degree of pleomorphism and nuclei atypia (5). Osteoclastic like giant cells's origin is still unclear. Although morphologically they resemble to osteoclasts, the origin of these cells seems to be histiocytic in origin as shown by immunohistochemical and ultrastructural studies (18). Thus, these cells may occur as a stromal reaction to tumoral cells and tumoral biochemical microenvironment.

In summary, tamoxifen may lead to stromal reactions as we have observed in this case. Osteoclastic like giant cells may occur in such patients. Atypical stromal cells were also present in the endometrium. Tamoxifen commitment may lead to the development of atypical stromal cells in the endometrial polyp and osteoclastic like giant cells in the leiomyoma. Clinicians should bear in mind that the above pathologic changes may occur as a manifestation of tamoxifen treatment. These patients should be followed meticulously to detect en-

dometrial and myometrial, tumoral, or proliferative lesions.

Ethics: The patient has been confirmed that written informed constent which include the case details.The patient also has confirmed about her published histopathological images.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

A case of Kallmann syndrome associated to a novel missense mutation of the FGFR1 gene

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Summary. *Background:* Loss-of-function mutations of fibroblast growth factor receptor 1 gene (*FGFR1*) have been reported so far. These mutations have been described in the extracellular domain, consisting of three Ig-like domains in the single transmembrane helix and in the intracellular region, containing a tyrosine kinase domain and cause about 10% of all cases of Kallmann syndrome. *FRGR1* mutations could be associated with non reproductive phenotype such as cleft palate and dental agenesis and a wide spectrum of reproductive phenotype. *Case Report:* The patient, 17 years and 11 months old, was a Bulgarian male referred to our Pediatric Endocrinology Unit for pubertal failure and hyposmia. Clinical evaluation revealed a highpitched voice, gynecomastia and obesity. Hormonal study revealed hypogonadotropic hypogonadism. Molecular analysis, performed by Next Generation Sequencing and confirmed by Sanger sequencing, led to the identification of a novel and previously undescribed mutation c.1058 C>G (p. S353C) in heterozygous state on exon 8 of the *FGFR1* gene. *Conclusion:* The novel mutation, that we found in a boy with Kallman syndrome, could destabilize the D3 immunoglobulin like receptor domain that is crucial for the FGF-FGFR interaction. (www.actabiomedica.it)

Key words: Kallman syndrome, mutation, FGFR1 gene

Background

Congenital hypogonadotropic hypogonadism (CHH) is a cause of pubertal failure. It is usually due to inadequate secretion of pituitary gonadotropins, while testicular/ovarian endocrine and exocrine functions are normal. When CHH is combined with anosmia or hyposmia, it is considered Kallmann syndrome (KS) (1). The KS prevalence has been estimated at 1/8000 in males and 1/40.000 in females (2) and it accounts for approximately 40% of the total CHH cases. It is due to an abnormal embryonic development of the peripheral olfactory system characterized by impaired olfactory neuron axon elongation and GnRH cells migration (3). We report a case of a boy with KS caused by a novel mutation on *FGFR1* gene.

Case report

A Bulgarian boy, 17 years and 11 months old, referred to our Pediatric Endocrinology Unit for pubertal failure and hyposmia. His stature: 171.6 cm (-0.69 SDS), upper segment: 84.3 cm, lower segment: 87.3 cm and arm span 180 cm. His weight was 92 Kg (1.83 SDS) with BMI of 31.24 kg/m^2 (2.22 SDS). Clinical evaluation revealed a high-pitched voice, gynecomastia, obesity and valgus knees. Penile length 3.2 cm, bilateral volume testes about 2 ml and pubic hair Tanner III stage. No evidence of dysmorphic features; he had a double upper urinary tracts. Hormonal basal study revealed low values of FSH (0.29 mUI/ml), LH (0.09 mUI/ml) and testosterone (0.2 ng/ml) for sex and chronological age. GnRH stimulation test elicited

a prepubertal LH response with LH and FSH peaks of 1.18 mUI/mL and 2.43 mUI/mL respectively. The karyotype was 46,XY. Brain magnetic resonance imaging was normal. A subjective smell test confirmed hysposmia. According to this clinical and hormonal picture we hypothesized a Kallmann Syndrome and started gonadotropin therapy. After obtained an informed consent, we performed an extensive NGS mutational analysis by a specific platform including the following genes: CHD7, DUSP6, FEZF1, FGF17, FGF8, FGFR1, FLRT3, GnRH1/2, GnRHR, HS6ST1, IL-17RD, ANOS1 (KAL1), KISS1, PROK2, PROKR2, SEMA3A, SEMA3E, SEMA7A, SOX2, SOX10, SPRY4, TAC3, TACR3, WDR11. The raw data for all of the above genes were then analyzed by filtering the sequence's variants using a specific mutational reference panel for each gene (1000 Genes Project, Roche illumina data bank). The *fgfr1 gene* gave the highest score, and the final Sanger sequencing confirmed the presence of a c.1058 C>G transition in heterozygous state located on exon 8 leading to a Serine to Cystine change never previously described.

Discussion

In this case, the presence of CHH in association with defective sense of smell allowed the clinical diagnosis of KS. KS is characterized by a variable degree of hypogonadism and olfactory dysfunction, among unrelated patients and within the same family (2). A subgroup of these patients can present a specific non-reproductive phenotypes, including involuntary upper limb mirror movements (bimanual synkinesis), congenital ptosis, abnormal eye movements, agenesis of the corpus callosum, hearing impairment, unilateral (occasionally bilateral) renal agenesis, cleft lip or palate, hypodontia and obesity (4). KS can occur sporadically or be hereditary. Hereditary cases have been well documented with different inheritance: X chromosome-linked recessive, autosomal dominant and autosomal recessive (5). Several genes are involved in etiology of KS: mutations in KAL1 gene cause X-linked forms; FGFR1, FGF8, CHD7, HS6ST1, SOX10, SEMA3A, WDR11 and IL17RD are correlated to autosomal dominant form; PROKR2, PROK2 and FEZF1 are described in association with autosomal recessive and oligogenic forms (6, 7). FGFs (fibroblast growth factors) and their receptors (FGFRs) comprise a large family of signaling molecules that have been shown to play crucial roles as growth factors in vertebrate and invertebrate embryonic development. FGFR gene family consists of four highly related genes (FGFR1-FGFR4) that have distinct binding affinities with FGF ligands. FGFR1 protein is a transmembrane receptor with an extracellular region of three immunoglobulin-like domains (8). In response to FGF stimulation a variety of signaling proteins are phosphorylated, leading to activation of downstream signaling pathways (9,10). In our case, molecular analysis led to the identification of a novel and previously undescribed mutation c.1058 C>G (p. S353C) in heterozygous state on exon 8 of the fibroblast growth factor receptor 1 gene. The in silico analysis demonstrated that the mutation is located on the D3 immunoglobulin like receptor domain that is crucial for the FGF-FGFR interaction. The mutation c.1058 C>G (p.S353C) determines a rearrangement of the H bond pattern with Thre 340 leading to a repositioning of the adiacent Tyr 339 (Figure 1). Based on this preliminary evaluation there could be a significative possibility that such change could destabilize the D3 domain, hampering its function and determining a lost of efficiency in the ligand receptor interaction.

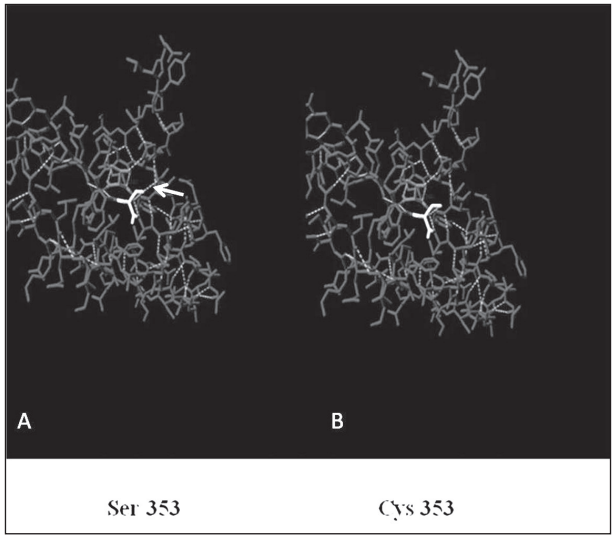

Figure 1. The mutation c. 1058 C>G (p.S353C) determines a rearrangement of the H bond pattern with Thre 340 leading to a repositioning of the adiacent Tyr 339

Experiments are in progress to prove this hypothesis. Our findings, expanding the number of the molecular defects on the FGFR1 responsible of KS, strenght the role of this gene in the pathogenesis of the disease and confirm the high molecular heterogeneity of this syndrome.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

A giant keratoacanthoma of the cheek

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Summary. Keratoacanthoma (KA) is a cutaneous tumor arising on sun-exposed skin and characterized by self-limiting growth and involution. We reported a case of a 92-year-old man presented a 4.5x3.5 cm nodular lesion with a central keratin-filled crater on his left cheek. We performed surgical excision and histopathological examination revealed a keratoacanthoma with perineural invasion. A close follow-up was carried out. (www.actabiomedica.it)

Key words: keratoacanthoma, skin cancer, squamous cell carcinoma

Introduction

The nonmelanoma skin cancers (NMSCs) most frequently diagnosed are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) (1).

Cutaneous SCC represents an uncontrolled growth of abnormal keratinocytes, arising on sun-exposed skin (2). The precancerous lesion is actinic keratosis (AK), a keratinocyte-derived precursor found predominantly in fair-skinned people, which can advance to SCC in situ, invasive SCC and finally to metastatic SCC (3).

Keratoacanthoma (KA) is a cutaneous neoplasia arising on sun-exposed surfaces and characterized by self-limiting growth and involution. The life cycle consists of three distinct stages (proliferative, stabilization and involutional) and takes about 4 to 6 months (4). The differential diagnosis between this neoplasia and SCC is a challenge due to their similar appearance.

Case Report

A 92-year-old man presented a nodular lesion with a central keratin-filled crater on his left cheek. His daughter reported it appeared about 2 months before as a small nodule and increased in size with fast growth. He was previously visited by a dermatologist who suggested surgical excision.

Physical examination revealed a 4.5x3.5 cm skin horn lesion: crateriform architecture filled with a keratin plug, which gave conical projection, no signs of inflammation in peripheral skin and painless (Figure 1a and 1b). There were no palpable regional limph nodes.

The lesion was excised under local anesthesia and the skin loss was repaired with local advancement flaps. Histopathological examination revealed a "keratoacanthoma-type squamous cell carcinoma" (or only "keratoacanthoma") with perineural invasion.

During postoperative follow-up of 6 months, no recurrence was noted (Figure 1c).

Discussion

Exophytic lesions with a central keratin-filled crater are difficult to diagnose clinically (5). Keratocanthoma and squamous cell carcinoma shares some features so they cannot be confidently differentiated by dermoscopy (6).

Figure 1. Preoperative and postoperative pictures. a) and b): Keratoacanthoma: a 4.5x3.5 cm skin horn lesion with crateriform architecture filled with a keratin plug and no signs of inflammation in peripheral skin; c: Postoperative image

Histopathological diagnosis allows to classify epithelial crateriform tumors into seven types: crateriform verruca, crateriform seborrheic keratosis, keratoacanthoma (KA), KA with a conventional squamous cell carcinoma (SCC) component (KA-like SCC and KA with malignant transformation), crateriform Bowen's disease, crateriform SCC arising from solar keratosis and crater form of infundibular SCC (7).

Whether KA is benign or malignant is controversial: it can be classified as a benign self-limited squamous proliferation (8), or as a type of well differentiated squamous cell carcinoma (SCC) capable of spontaneous regression (9).

KA is usually solitary but can be multiple (10). It is assumed to originate from the hair follicle: KA exhibits markers corresponding with those found in the follicular isthmus and infundibulum. Its life cycle with proliferative, mature and involutional phases mimics the hair cycle (11).

Perineural invasion of keratoacanthoma is rare (12). Keratoacanthomas of head and neck with perineural invasion have a greater potential for aggressiveness: their spread into the mimic muscles, cranial nerves or sinus cavernosus, local recurrences, metastases in the parotis gland and regional lymph nodes have been reported in literature (13). When perineural invasion is extended, the prognosis can correspond to that of squamous cell carcinoma with perineural infiltration, so a closer follow-up of the patient is recommended (12-14).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

Non artheritic bilateral anterior ischaemic optic neuropathy (NAION) as devastating complication following Total Hip Arthroplasty: a case report

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Summary.*Introduction:* Postoperative vision loss (PVL) is an extremely rare complication following major surgical procedures. Patients with systemic hypertension, diabetes, coronary diseases and smokers are generally predisposed to this complication. More frequently, it is caused by ischemic optic neuropathy (ION), central retinal artery occlusion or retinal vein occlusion. Rare cases of unilateral PVL following total joint arthroplasty surgery have been recently described in literature. *Case report:* This case report describes the first reported bilateral non-arteritic anterior ischemic optic neuropathy (NAION), which occurred 3 days following a total hip arthroplasty with a consequent post-operative hypotension. *Conclusions:* Orthopedic surgeons should be aware that in hip joint replacement procedures, selected patients present an higher risk of ION following intra/postoperative hypotension and prolonged surgical times. (www.actabiomedica.it)

Key words: bilateral visus loss, total hip arthroplasty, case report

Introduction

Postoperative vision loss (PLV) is a severe non-ocular surgical complication resulting in permanent vision loss (1). The occurrence of PVL in general surgery is very rare (0.0008%) with an higher incidence in selected procedures, such as spine surgery (0.2%) or cardiac surgery (0.11%). Only few cases have been reported following elective orthopedic procedures (2). PVL onset varies from the immediate post operative time to 10 days after surgery with an average onset time of 15 hours (0-168 hours). The most frequent causes of PVL are ischemic optic neuropathy (ION), central retinal artery occlusion (CRAO) and retinal vein occlusion (RVO) (3-5). Newman et al. identified several risk factors for bilateral sudden visual loss following non-ocular surgeries including diabetes mellitus, systemic hypertension, perioperative hypotension, acute anemia due to excessive blood loss, excessive liquid replacement, vasoconstrictive agents, and supine position during surgery (6).

Likewise nowadays, in accordance with the cost-saving procedures advocated by many health organization, several authors have debated the potential advantages of deliberate hypotension during anesthesia for selected surgical procedures to reduced intraoperative blood loss and facilitates an earlier uncomplicated patient discharge (7, 8) despite more frequently surgeons have to face older patients often affected by significant comorbidity (9).

In the literature, we could find very few reports dealing with unilateral PLV following hip surgery (10-

13). To our knowledge, there is just one case of a bilateral PLV managed in an Ophthalmologic center and published in a non-orthopedic journal without either mention about orthopedic management or final visual loss prognosis (14).

Our case report aims to document the clinical history of a patient, who suffered a permanent bilateral visual loss because of a non-arteritic anterior ischemic optic neuropathy (NAION) three days later after a total hip joint arthroplasty.

Case report

A 63 years old man, with BMI of 38, a history of unstable diabetes mellitus, high blood pressure, dyslipidaemia and hyperuricaemia was referred to our department to receive an uncemented total hip arthroplasty due to an unilateral severe coxarthrosis (grade IV according Kellgren-Lawrence system) (15) (Fig. 1).

Seven months before surgery, an ophthalmologic examination showed a corrected visual acuity (BCVA) of 20/20, normal intraocular pressure with no signs of diabetic retinopathy at fundus inspection. Preoperative haemoglobin (HGB) value was 16.7 g/dL and haematocrit (HCT) was 49.2%.

The patient underwent to a spinal block (ropivocaine HCL+ sufentanil) supplemented by a mild sedation (midazolam) to receive a total hip arthroplasty on a supine decubitus using a direct lateral approach. Antibiotic prophylaxis (cefazolin) was given 30 minutes before skin incision according to the departmental protocol. The surgery was complicated by an intraoperative femoral stem exchange to an higher off-set design because of an intraoperative joint instability ending in total surgical time of 170 minutes. Intraoperatively a mild hypotension was strictly controlled with intravenous fluids (4000 ml) and at the end of surgery, the patient was in good condition with a good pain control and a blood pressure of 100/60 mmHg. During the first night, the patient suffered of an episode of hypotension (70/40 mmHg) associated to diaphoresis, both corrected using crystalloids infusion (3000 ml) and the following morning the blood pressure was 90/70 mmHg with a value of 11.2 of HGB. During the first 2 days after surgery, he had an average blood pressure, HGB and HCT of 100/70 mmHg, 10,1 g/dL and 30.2, respectively. The drain was removed 48 hours after the operation and the patient gradually started the physiotherapy program. Three days after surgery, he complained a sudden bilateral visual worsening with a BCVA of 1/40 in the right eye and of 1/20 in the left eye. Blood pressure, HGB and HCT values were 105/75 mmHg, 9.5 g/dL and 26.2% respectively. He was unsuccessfully investigated with a CT of the brain and an echo US-Doppler of the supra-aortic vessels. The funduscopic exam showed an oedematous optic nerve with a peripapillary haemorrhage in the right eye and a pale optic disc in the left eye. The patient underwent to both a Optical Coherence Tomography (OCT) and fluoroscopy with evidence of bilateral papilledema, peripheral hypoperfusion, and posterior pole leakage (Fig. 2). OCT showed diffuse thinning of the Retinal Nerve Fiber Layers (RNFL) and a diag-

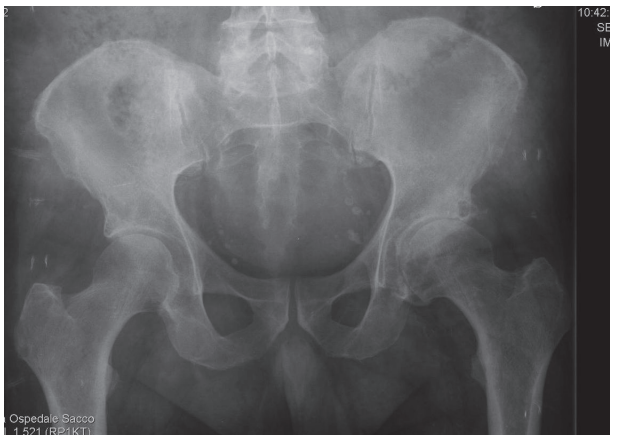

Figure 1. Preop and at 1 year follow-up AP images of the hip

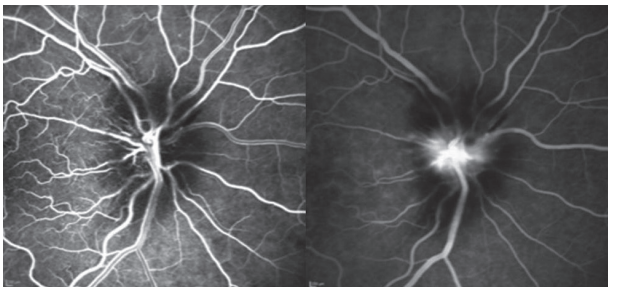

Figure 2. Fluorescein angiography with both early and late phases showing an oedematous optic nerve head with leakage in the late phases

nosis of a feasible bilateral NAION was consequently formulated. According to the international guidelines, the acute ophthalmological treatment was based on high dose of corticosteroid (60 mg/dl/day) for the first 5 days with a maintenance dose (20 mg/dl/day) for further 30 days (3). Intraocular hypotonic eye drops were administrated to modulate the 18 mmHg Intraocular Pressure (IOP).

Moved to hospital's rehabilitation department, he was discharged after 45 days and he was able to walk with a full weight-bearing using two crutches but without any sign of improvement in visual loss. The patient was checked every 2 months and at 1-year follow-up he was able to walk independently with pain-free and almost complete recovery of the hip ROM. Because of a pale papilla, the situation is now stable without any improvement regarding the bilateral visual loss.

Conclusions

In Orthopaedic surgery there are extremely rare reports of sudden perioperative vision mainly occurring few hours after surgery and mostly caused by ischemic optic neuropathy (ION) (1-4)

Patients with systemic hypertension, diabetes, coronary diseases and smokers have a higher risk of postoperative ION (2, 5). Cho et al. advised the orthopedic community about ION risk after uncomplicated hip surgery in elderly patients following femoral neck fracture surgery (12). Buono et al. reported a very poor visual prognosis in ION following non-ocular surgery, recommending a mandatory prevention (4).

In a literature review published in 2011, Kaeser et al. reported 4 unilateral visual loss cases following hip arthroplasty, although none of them caused by a non-artheritic anterior ischemic optic neuropathy (NAION) (10). Only in 2014, Yazgan et al. described a simultaneous bilateral NAION following unilateral hip arthroplasty (14). In this case the patient was referred to an ophthalmic centre with no precise data regarding intra and perioperative pressure management (13). Furthermore, at the time of the ophthalmic examination, many hours after the surgery, the patient was still anaemic (HBG:7.8 g/dl) making the blood loss and hypotension management questionable. Likewise visual and orthopaedic prognosis were not mentioned because patient gave up any further follow-up control (14).

Our case is the first simultaneous bilateral NAION following hip arthroplasty submitted to a scientific journal with a complete 1-year follow-up. The patient referred the visual loss onset after 3 days postoperatively despite we constantly monitored blood pressure and blood values to restore any hypotension. We observed that even a temporary mild hypotension could cause an ION occurrence even in relatively young age but complicated by some risk factors (type 2 diabetes mellitus, systemic hypertension). Furthermore we should have consider a prolonged surgical time with a consequent increased blood loss as a contributor to the first post-operative night hypotension. In literature several authors encourage a controlled hypotension in joint arthroplasty surgery remarking the potential advantages for both surgeons and patients in terms of blood loss, easier surgeries and faster discharge in accordance of a new "fast track" patient management policy even in bilateral implants(7, 8). Nevertheless nowadays surgeons have to face more often demanding surgeries either in older pluri-pathological patients or in complicated multiple hip revision procedures all requiring an accurate intra/preoperative blood and pressure management without considering any priority to an earlier discharge (9).

The aim of this case report is to advise both surgeons and anesthesiologists that ION can occur in hip replacement procedures with prolonged surgical times in selected patients suffering intra/perioperative hypotension. Patients affected by diabetes, hypertension, coronary diseases and smokers should be considered as high risk patients requiring both intra/post-operative pressure and blood loss accurate management even considering longer hospital staying.

Conflict of interest: None to declare

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Case report

Recurrence of tumoral calcinosis: a case report

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Summary. We describe radiographic, contrast-enhanced MDCT and MRI findings with pathologic correlations of an unusual recurrence of tumoral calcinosis, also called Teutschlander disease. The disease was silent in the first decade of life, when it appeared with elbows recurring lesions, until the seventh decade of life, when a left hip active growth lesion developed. A review about tumoral calcinosis pathogenesis, clinical course and imaging differential diagnosis is reported. (www.actabiomedica.it)

Key words: calcifications; tumoral calcinosis, Teutschlander disease, musculo-skeletal (MSK) imaging, magnetic resonance imaging

Introduction

Tumoral calcinosis (TC), also called Teutschlander disease, is a relative rare disorder characterized by calcium salts accumulation in iuxta-articular soft-tissues, producing solitary or multiple painless periarticular masses (1, 2). Large joints such as the hip, shoulder, and elbow are usually involved. This entity most commonly presents in the first 2 decades of life. Approximately one-third of patients with TC shows familial inheritance (3, 4).

TC has been generally presented as case reports and the plain radiography findings of the disease have been well documented. To date, there are few studies presenting computed tomography (CT) and magnetic resonance (MR) imaging characteristics of TC with radio-pathologic correlation (5).

We report the unusual case of an adult patient without familiar inheritance, who had undergone surgery for elbows TC during the first decade of life and presented left hip recurrence in the sixth decade. We describe CT and MR findings with histopatological correlation on surgical specimen.

Case report

A 64 years-old male patient was referred to our institution for swelling of the left gluteus and hip. He had a history of elbow recurring TC in the first decade of life that needed five surgical procedures. No other manifestations of calcinosis occurred during the following decades. He didn't referred TC in the other family members.

The patient had a mild hyperphosphatemia, without haematic value alterations of 1,25-dihydroxyvitamin D or parathyroid hormone. Renal function was normal.

Radiography showed a grossly periarticular calcified mass around the left hip joint and the upper thigh (Fig. 1). At contrast-enhanced (CE) multidetector computed-tomography (MDCT) a grossly calcified

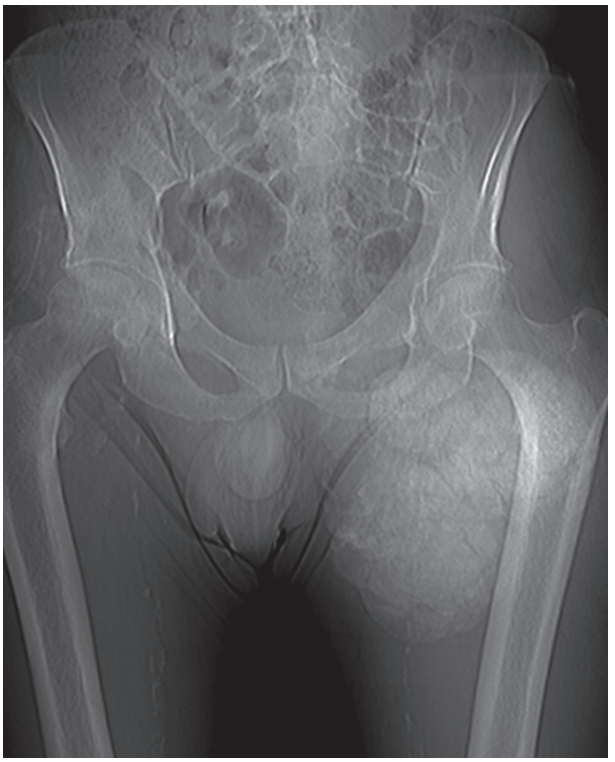

Figure 1. Radiography shows a grossly periarticular calcified mass around the left hip joint and the upper thigh

lobular mass was visible with large cystic areas and fluid-fluid levels inside (CT sedimentation sign) (Fig. 2a and 2b). A peripheral enhancement of cystic areas was observed after CE administration, without pathologic solid portions. The mass was localized in the region of the left greater trochanteric bursa, deeply to gluteal muscles, extending medially to the upper part of the ischio-pubic branch, and along the posterior compartment of the lower part of the thigh, as well demonstrated by CT multiplanar and tridimensional (3D) images (Fig. 3a-c).

At MRI the lesion had inhomogeneous diffuse low signal intensity on T1-weighted sequences, while it presented alternating signal patterns on T2-weighted sequences with low intensity areas and cystic components with fluid-fluid levels (MRI sedimentation sign) (Fig. 4a-f). After CE administration, fibrous septa surrounding cystic and calcified areas enhanced, producing a "web" or "cobblestone" pattern. No nodular enhancement was reported, neither involvement of surrounding muscolar and bone structures, vessels and nerves.

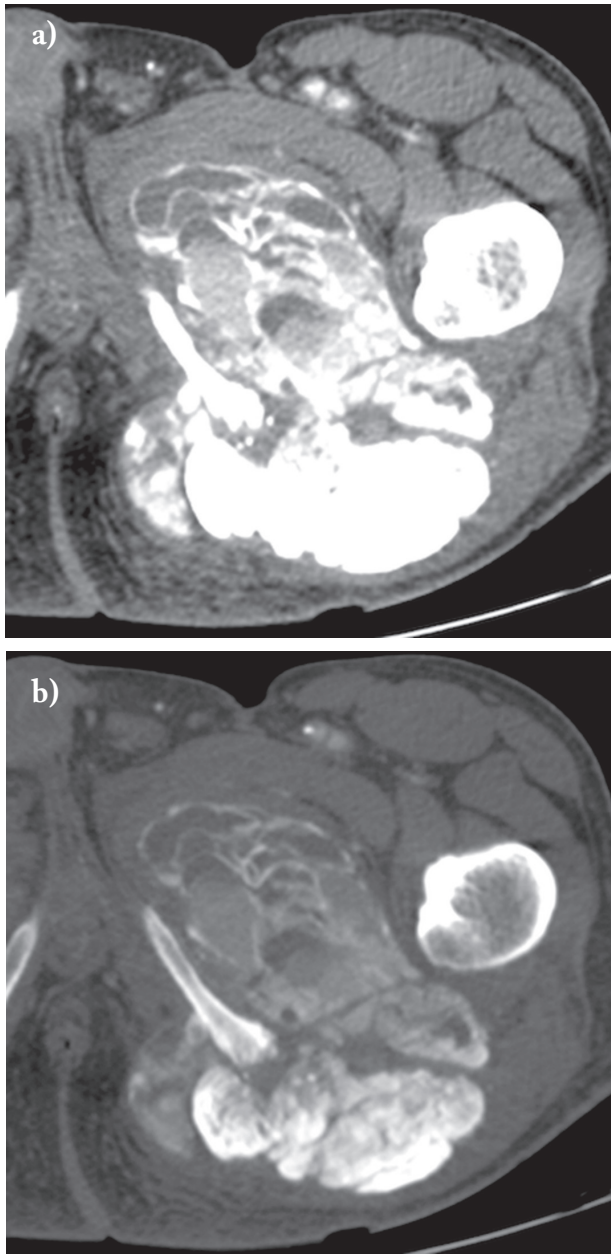

Figure 2. On MDCT, reported in soft-tisse (**a**) and bone (**b**) windows, the mass has large cystic areas, with gross and egg-shell calcifications and fluid-fluid levels inside ("sedimentation sign")

Patient underwent surgery. The mass was resected, measuring 62 cm in the largest diameter. Pathologic evaluation confirmed the diagnosis of TC, showing grossly calcifications and cysts with chalky material inside (precipitated calcium salt), surrounded by inflammatory reaction and fibrosis (Fig. 5a-c). No signs of

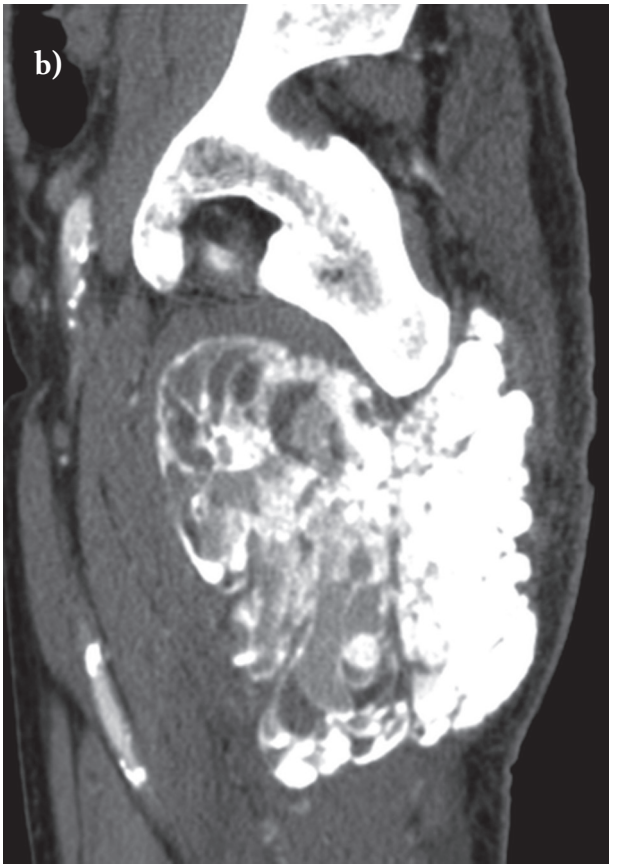

Figure 3. MPR reconstructions on coronal (**a**) and sagittal (**b**) planes and 3D reconstructions (**c**) CT scans better depict lesion localization and extension around the left hip joint and the upper thigh

malignancy were found and no part of the mass was seen to make direct contact with the underlying bone.

Discussion

TC was first described by Giard (7) and Duret (8) in 1898 and 1899 and then widely studied from 1930 to 1950 by a german pathologist, Otto Teutschlander and named it as "lipocalcinogranulomatosis" (9). The current term of TC was firstly used by Inclan in 1943 in American literature (10). During the following years the disease has been identified with different terms and criteria. To date, approximately 200 cases have been reported since Duret's first description in 1899 (11-14).

Pathogenesis of TC is not known, and similar lesions can be observed in metabolic disease with increased haematic levels of calcium and phosphate, such as chronic renal failure, primary hyperparathyroidism, hypervitaminosis D, sarcoidosis (15, 16). On the basis of 122 reviewing cases and their clinical and pathologic findings, Smack et al divided all TC in three groups (17). One group are primary normo-phosphatemic TC usually presenting in first two decades of life as solitary lesions. According to this group of authors these patients are without evident familial connection, although recent literature detected familial connection involving mutations in the gene encoding SAMD9 protein. The second group are primary hyperphosphatemic TC usually presenting during the first and second decades of life. This group of patients have genetic predisposition with reduced urinary phosphate excretion caused by recessive mutations in *GALNT3* and *KLOTHO*, that causes the inactivation of FGF23, a phosphaturic hormone. The third group encompasses secondary TC connected with chronic renal failure. Despite their different etiology and pathogenesis, histopathology is identical in all types suggesting possible common path-way which eventually results in the for-

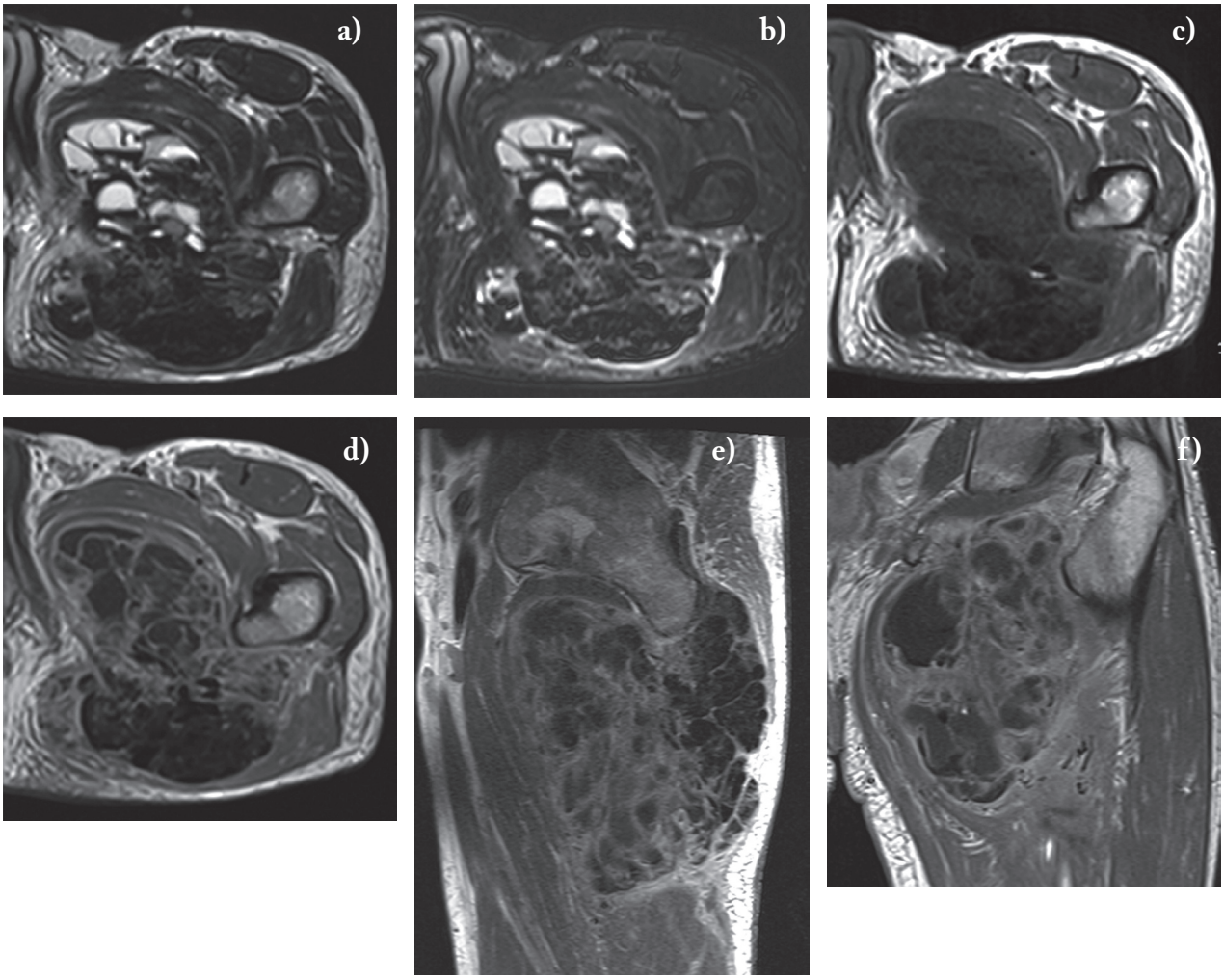

Figure. On MRI axial T2w (**a**) and fat-suppressed T2w (**b**) sequences the lesion present low intensity and bright nodular areas, with cystic components and fluid-fluid levels ("sedimentation sign"). On T1w sequences (**c**) lesion shows inhomogeneous diffuse low signal intensity. After CE injection, T1w axial, coronal and sagittal images show (**d, e** and **f**) fibrous septa surrounding cystic and calcified areas enhanced, producing a "web" or "cobblestone" pattern.

mation of the characteristic TC lesions and originates from minimal repetitive trauma and reparative inflammatory process (17-20).

Typical radiological findings in TC suggest a correct diagnosis in the majority of cases. Radiographs show lobulated calcifications on joints extensor surface. Calcifications are well defined, rounded, sometimes with a cystic appeareance and a fluid-fluid level inside (14). Fluid-fluid levels, producing the "sedimentation sign", derive by calcium crystals layering inside intracystic amorphous chalky material. Fibrous septa can produce linear or curved radiolucencies between gross calcifications, producing a "cobblestone" pattern (12).

CT shows calcified cystic lesions in fibrofatty planes, deeply to muscles. It clearly depicts lesion topography and extension, particularly using 2D Multiplanar Reconstruction (MPR), 3D Shaded Surface Display (SSD) and Volume Rendering (VR) reconstruction. Reconstructions help surgeons to correctly plan a complete surgical ablation (1).

At MRI lesions have variable signal intensity, with a low signal in largely calcified portions, and cystic areas with high signal in T2-weighted sequences. At MRI the typical "sedimentation sign" shows a greater

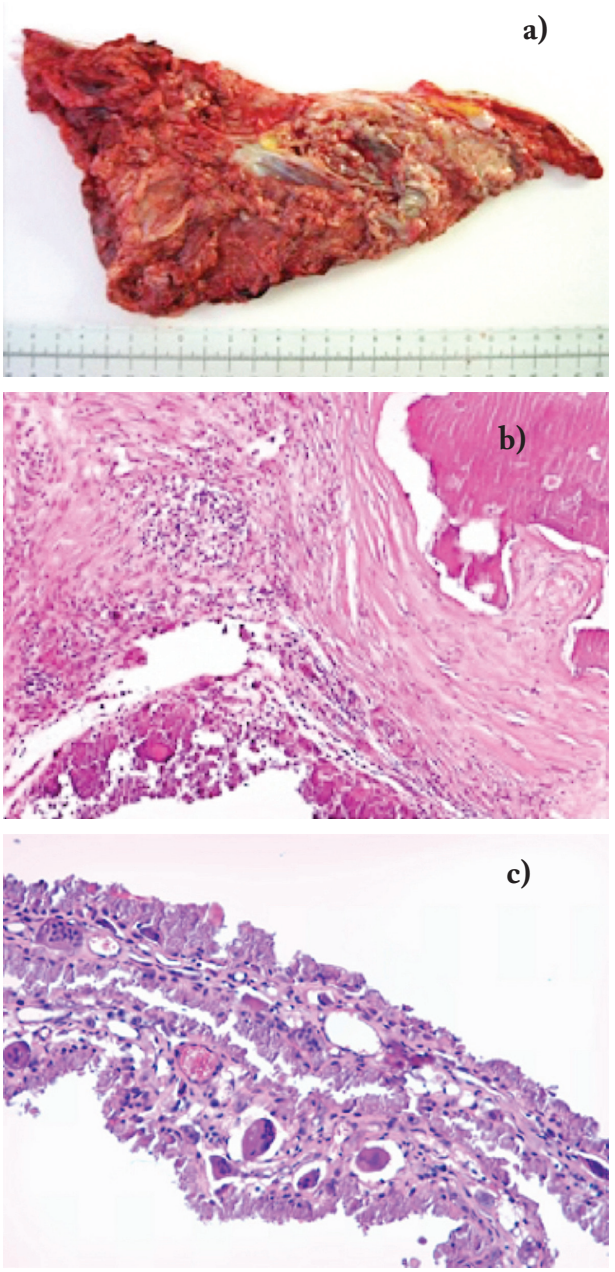

Figure 5. (**a**) The cut surface of the surgical specimen was yellow-white with a cystic appearance. (**b**, **c**) Low-power photomicrographs show calcific debris with chronic inflammatory reaction and surrounding fibrosis.

conspicuity (5). According to some authors (1), an alternating high and absent signal MRI pattern is associated to metabolically active lesions, with a prominent fluid/inflammatory component. After contrast injection, only a mild contrast enhancement of fibrous septa can be observed (21).

Some authors have documented an ultrasonographic (US) approach to TC (22, 23). In acute cases, in which a mass is initially forming or beginning to enlarge and vascular signals are more detectable, TC may present as a well-circumscribed homogenous mass with an irregular border of calcifications. In chronic cases, which tend to be defined by more cystic changes and fibrous septa, US may reveal a lobulated cystic echogenic mass with septa and a calcified rim. The sedimentation sign may also be observed (22-26). However, the cystic appearance on US is aspecific since it may easily mimic as an abscess formation (27, 28).

Also the value of angiography in evaluating soft tissue calcific masses has been reported (29-33). Benign soft tissue masses do not typically change the normal vessel diameter, nor do they demonstrate neovascularity, blush, pooling, encasement, arteriovenous shunts or feeding vessels (34, 35). Angiography of benign soft tissue masses typically shows displaced or stretched vessels (36). TC is atypical for benign soft tissue masses in that it is a relatively vascular process. In a radio-pathologic correlation study (37), TC has been proven to be hypervascular during the active phase, similarly to myositis ossificans. Specifically, the active stage of tumoral calcinosis has fluid with sedimentation or calcific foci at the edges, cellular pleomorphism and a prominent vascular component (36, 37).

This active and hypervascular stage could take advantage of an early surgery (29). In this setting, some authors have proposed a vascular study of TC lesions by contrast-enhanced ultrasound (CEUS), which is a "new" simple, immediate, and effective US tool: microbubbles circulate freely inside the body and constitute an intravascular contrast agent (38, 39); therefore, they permit analysis of both macro- and microvascular lesional blood flow (40, 41). The technique yields information about contrast enhancement almost as CT (42, 43) and MRI (44, 45) do but in real time and without the use of ionizing radiation. To date, CEUS has obtained valid results in different areas (38-41), although the method has not yet entered in standardized vascular imaging protocols for clinical practice.

Differential diagnosis includes many conditions with similar periarticular soft tissues calcifications. Grossly periarticular soft-tissues calcifications can be observed in connective tissue diseases (polymyositis,

dermatomyositis, lupus erythematosus) or traumatic/degenerative diseases (synovial osteochondromatosis, calcific tendonitis, myositis ossificans, calcific myonecrosis). Clinical history, joint involvement and morphology and distribution of calcifications usually allow a correct differential diagnosis (46, 47). Differential diagnosis with malignant neoplasms is critical, particularly with synovial sarcoma, parosteal osteosarcoma and chondrosarcoma. Synovial sarcoma usually develops in periarticular regions as an inhomogeneous infiltrative mass only partially occupied by grossly calcified areas inside (48). Parosteal osteosarcoma and chondrosarcoma arise from bone surface extending in surrounding soft tissues as a densely calcified mass. Radiographic appeareance may suggest TC when bone don't show significant anomalies. Cross-sectional imaging accurately depicts sarcomatous malignant infiltrative growth toward underlying bone and surrounding structures and a high enhancing tissutal portion after CE inside the mass or around calcified components (1).

In the case presented the lesion had typical radiological features of TC. The mass was localized at the extensor surface of hip joint, with a benign expansive growth. It was largely occupied by calcifications and cystic areas with fluid-fluid levels inside ("sedimentation sign"); no nodular enhancing areas were evident. MRI also showed an inhomogeneous high signal intensity in T2-weighted sequences, although these features have been more frequently reported in younger patients with metabolically active lesions (1). Moreover, the disease showed an unusual course: after a typical onset in the first decade of life, disease had been silent until the sixth decade, when it recurred with a left hip active growth lesion.

Surgical excision is a well documented treatment, but recurrences after surgery due to poor circumscription are common, particularly in metabolically active lesions (5). Recurrences can also occur several years after intervention (49). Until now, however, only few reports in literature have described MRI findings in TC recurrences (5).

To conclude, TC can occur with different clinical and biochemical patterns. We report an unusual relapse with a metabolically active lesion in an adult man after a long period of quiescence. Differential diagnosis can be difficult in unusual clinical settings, but a careful evaluation of imaging findings and biochemical data can suggest the correct diagnosis.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

Hemobilia due to cystic artery pseudoaneurysm following cholecystectomy: diagnosis and management, a case report

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Summary. Hemobilia is an unusal cause of upper gastrointestinal bleeding and may be the result of the formation of an hepatic vessel pseudoaneurysm. This is a rare occurence after laparoscopic or open cholecistectomy. The most importants factor for pathogenesis are direct or indirect iatrogenic injuries during intervention and hepatic trauma. Clinical presentation may also be late and includes more frequently upper gastrointestinal bleeding due to pseudoaneurysm rupture, abdominal pain and jaundice secondary to bile duct compression. Therapies includes trans arterial embolization of feeding artery and percutaneous ingjection of embolic devices into the aneurysm. Surgery must be reserved for cathether based therapy failure. We report a case of a 66 year old man, presenting a month after cholecystectomy, complaining abdominal pain in the upper right quadrant and hematemesis. An EGDS exam showed hemobilia and computed tomography (CT) revealed a cistic artery pseudoaneurysm (PSA) wich have been successfully treated with hyperselective arterial embolization. Although this is a rare complication the surgeon must be aware of related symptoms and signs in order to sospect pseudoaneurysm as prompt recognition and treatment are essential. Untreated haemobilia may determine an immediate threat to life leading to acute haemodynamic instability We describe both diagnostic features and therapeutic strategies in comparison to the most recent literature. (www.actabiomedica.it)

Key words: hemobilia, pseudoaneurysm, cholecystectomy, arterial embolization

Introduction

Post-operative Hemobilia due to pseudoaneurysm is a rare but well-known occurrence representing only 6% of all causes of upper gastrointestinal bleeding. Artery pseudoaneurysm is a continuous inflammatory process that leads to erosion in the elastic and muscular components of the arterial wall, ultimately resulting in pseudoaneurysm formation. The most importants factor for pathogenesis are direct or indirect iatrogenic injuries during intervention and hepatic trauma.

Clinical presentation may also be late and includes more frequently upper gastrointestinal bleeding due to pseudoaneurysm rupture, abdominal pain and jaundice secondary to bile duct compression (3-12). Therapies includes trans arterial embolization of feeding artery and percutaneous ingjection of embolic devices into the aneurysm. Surgery must be reserved for cathether based therapy failure (13-14). We report a case of a 66 year old man, presenting a month after cholecystectomy, complaining abdominal pain in the upper right quadrant and hematemesis. An EGDS exam showed hemobilia and computed tomography (CT) revealed a cistic artery pseudoaneurysm (PSA) wich have been successfully treated with hyperselective arterial embolization. Although this is a rare complication the surgeon must be aware of related symptoms and signs in order to sospect pseudoaneurysm. We describe both diagnostic features and therapeutic strategies in comparison to the most recent literature.

Case report

I.C., 66 years old, chronic heavy smoker, with a history of hypertension, recently subjected to pace

maker implantation for atrial fibrillation, presented to our clinic for symptomatic cholelitiasis. US performed in previous months demonstrated multiple subcentimeter gallstones without any signs of acute cholecystitis. The patient had been subjected to Endoscopic retrograde cholangiopancreatography (ERCP) for choledocholithiasis. According to the program we performed a video laparoscopic cholecystectomy. During the surgical procedure an anomalous biliary duct for VI-VII hepatic segment, wich flows directly into the common hepatic duct over cystic duct is accidentally damaged. We therefore decided to convert the procedure from laparoscopic to open cholecystectomy. We also placed a Kehr type drain into the common hepatic duct from wich came some gallstone. Intraoperative cholangiography didn't show sign of bile spillage. Post-operative course was completely normal and the patient has been discharged 4 days later. Two weeks after we performed another cholangiography wich showed no signs of leakage.

About one month after the patient presented again to our clinic with a history of hematemesis, melena and upper and right abdominal pain from 2 days. The abdomen resulted distended and the patiens was pale. When the patient arrived at hospital, his blood pressure was approximately 80/60 mm Hg and his pulse rate was 110 beats per minute.

We decided to perform an endoscopic exam wich showed clots and blood in descending duodenum, and in particular showed a clot exiting through the major duodenal papilla (Fig. 1-2).

The patient has been hospitalized for hemobilia. Laboratory investigations revealed a significant fall in hemoglobin values (7.0 mg/dl). Other significant laboratory studies revealed the following values: white blood cell count, 18.3 × 10^{3}/uL; total bilirubin, 2,4 mg/dl.

A CT-SCAN analysis showed an oval blushing of contrast (10-11 mm)in arterial phase, next to a clip on hepatic pedicle as ay be seen in pseudoaneurysm. In delayed phase along the common biliary duct the CT-SCAN showed hyperdense material probably due to hematic losses. Hemobilia and dilatation of biliary ducts are also highlighted.

We decided to manage the PSA with a celiac trunk selective arterial embolization via right femoral catheterization. Angiography showed the PSA located

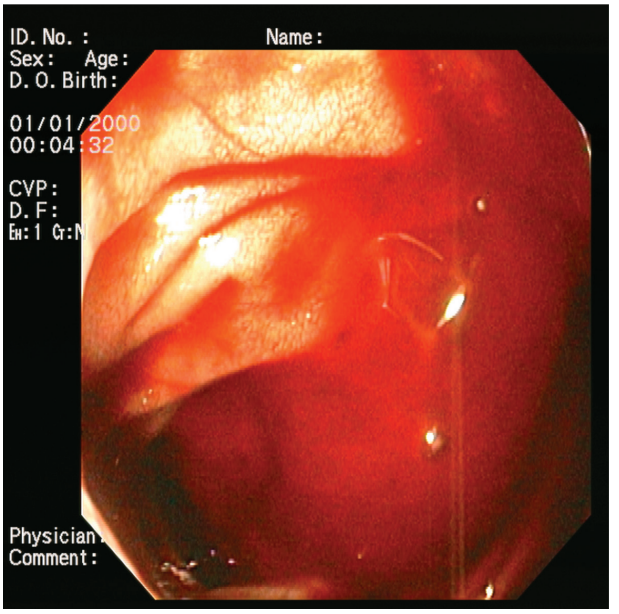

Figure 1. Bleeding from duodenal tract

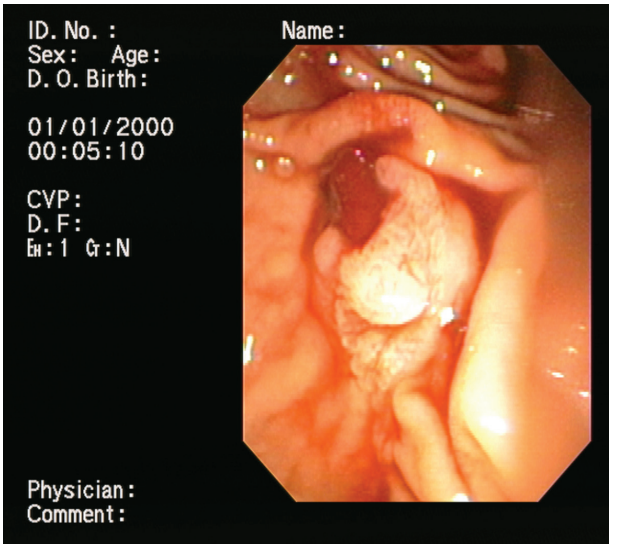

Figure 2. Bleeding from Vater's Papilla

near the the surgical clip, close to hepatic artery bifurcation (Fig. 3). Through superselective cathterization we placed multiple spiral coils (MRI compatible) inducing PSA occlusion (Fig. 4).

In post procedural course we infuse two concentred herytrocytes bags with hemoglobin value raise to 9.0 mg/dl the day after and to 10 mg/dl three days after. The patient has been discharged three days after hospitalization in good conditions.

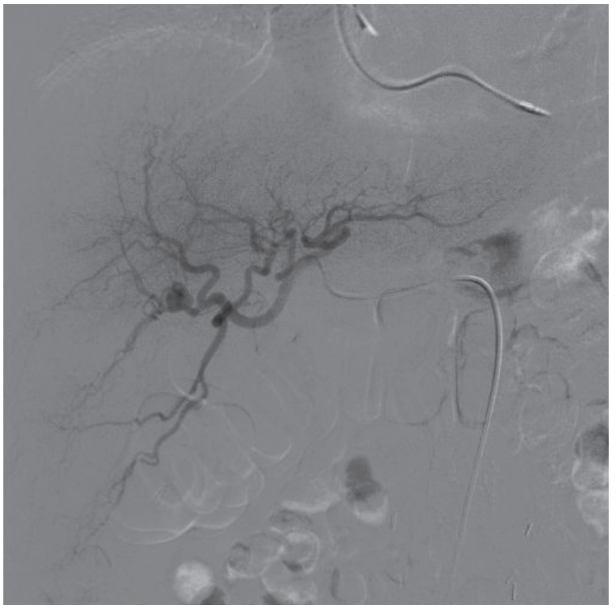

Figure 3. Cystic artery pseudoaneurism

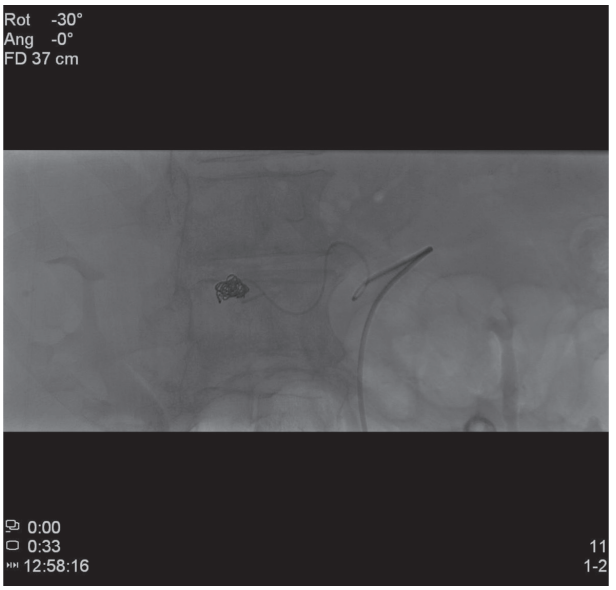

Figure 4. Embolization of pseudoaneurysm

The patient presented to our clinic a week after, totally asymptomatic, showing a normal hemoglobin value (11.4 mg/dl). An imaging guided follow up has been performed a month after using color doppler ultra sound that has not highlighted abdominal collection nor bile ducts dilatations.

Discussion

Hemobilia was first reported by F. Glisson in 1654 and the defined by Sandblomm in 1948 as an hemorrage into the bilary tract from a passageway between blood vessels and bile ducts (7). The most frequent causes of this pathological condition are liver traumas and pseudoaneurysm of epathic arteries. Less frequently inflammatory conditions or neoplasms are involved in the ethiology. Iatrogenic causes are nowadays the most important factors (two-thirds of cases are iatrogenic), procedures as hepatobiliary surgery, laparoscopic cholecistectomy, biliary drainage and liver biopsy may be complicated by the formation of pseudo aneurysm. Vascular damages and thermal injuries during laparoscopic Calot's triangle dissection are predisposing factors. However most cases described have a history of difficult or prolonged intervention (1, 5). Most likely, precipitating factors in the exposed case, include initial clip encroachment of the vessels, thermal or mechanical injury, and inflammation due to surgery.

The incidence of pseudoaneurysm formation is hard to determine , as asymptomatic aneurysm could not be easily determined or may thrombose spontaneously, while the risk of rupture is related to sizes (5, 9).

The time interval between procedures or surgery and onset of clinical symptoms is variable. Most of patients present a month after surgery but 5 years delayed presentation has been described.

Most of patients present with the classical symptoms: jaundice, biliary colic or upper abdominal pain, and gastrointestinal bleeding, but less then 40% patients present the complete triad first described by Quincke in 1871 (8, 9). In our case upper gastrointestinal bleeding from resulted from the cystic artery pseudoaneurysm's communication with the cystic duct.

Diagnosis is usually made with gastrointestinal endoscopy wich can demonstrate blood flow from duodenal papilla, if this procedure fails to show bleeding sources, urgent CT scan should be considered. Endoscopic retrograde cholangiopancreatography (ERCP) eventually followed by stent placement or sphincterotomy, is highly effective in diagnosing this threatening complication, giving possibility to control possible cystic duct stump leaks and treating obstructive jaundice.

CT scan may show abdominal collection, biliary tree dilatation, gastrointestinal distension, pleural effusion and, overall, suspected vascular abnormality, while catheter arteriography is used for therapeutic procedure as pseudoaneurysm or feeding vessel embolization. This procedure, performed with coils, gel foam thrombin and other agents, has replaced the surgical management of pseudoaneurysm wich requires aneursym resection and ligation of cistic artery. While transarterial embolisation is the treatment of choice for haemostasis, with a 75% to 100% success rate, surgery remains however the next step after an embolization failure (13,14).

Conclusions

In conclusion, patients who underwent laparoscopic cholecistectomy should be observed in post-operative course as cystyc artery pseudoaneurysm occurs as a rare complication with clinical symptoms often delayed in time. Although this is a rare complication the surgeon must be aware of related symptoms and signs in order to suspect pseudoaneurysm as prompt recognition and treatment are essential.

Untreated haemobilia may determine an immediate threat to life leading to acute haemodynamic instability, necessitating detection, access, and control of the vascular anomaly.

The suspect of PSA should be posed in symptomatic patients showing hemobilia, using endoscopy and imaging guidance as CT angiography. Therapy should be at first endovascular as more invasive surgical treatments may be used only after embolization failure.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

Acute abdominal pain in an adolescent girl with an ovarian yolk sac tumor

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Summary. Yolk sac tumor (YST) is a rare tumor that usually occurs in the first two decades of life. It is considered the second most common malignant germ cell tumor of the ovary, characterized by a rapid growth and a bad prognosis due to the frequent metastasis. We report the case of a 12-year-old girl who came to our observation for an acute abdominal pain. Clinical examination evidenced a vague mass in the suprapubic region and a lower abdomen tenderness, the US imaging revealed a complex lesion of the left ovary (19 x 13 cm) and the alpha-fetoprotein (AFP) resulted high (5858 ng/mL). Computed tomography (CT) revealed a large pelvic mass. The treatment consisted of debulking surgery of yolk sac tumor followed by 4 cycles of BEP protocol (Bleomycin, Etoposide, Cisplatin). After 3 years of follow-up there was no evidence of disease recurrence. (www.actabiomedica.it)

Key words: yolk sac tumor, germ cell tumor, ovarian torsion, ovarian tumor, alpha-fetoprotein

Introduction

Yolk sac tumor (YST) of the ovary, also known as endodermal sinus tumor (EST), is a rare malignant lesion, which belongs to the group of malignant ovarian germ cell tumors (MOGCT). It is considered the second most frequent MOGCT after dysgerminoma, with an incidence of approximately 1% of the ovarian tumors in children and adolescent (1, 2). It is highly malignant and it has a bad prognosis (3). Its frequency is higher in Japan compared to North America and Western Europe (4).The histology of the ovarian tumors is variable and the prevalence of the different types of tumors is different between adults and children (5). The incidence of malignant lesions increases with patient's age and tends to be associated with higher levels of serum tumor markers (6). So it is imperative that these lesions are precociously detected and effectively treated.

We present a case of a YST in a 12-year-old adolescent girl, remarking the importance to consider these rare tumors when an abdominal or pelvic mass is found.

Case Report

A 12-year-old Caucasian girl presented to the Emergency Department for a sudden abdominal pain. There was no known previous medical history. At clinical examination she was in Tanner's stage 2-3, afebrile, tachycardic, tachypneic and normotensive.

Abdominal evaluation revealed a swelling mass characterized by hard consistency and undefined outlines in the suprapubic region. A transabdominal ultrasonography (US) showed a heterogeneous mass of 19 x 13 cm with mixed echogenic and hypoechoic components. A pelvic computed tomography (CT) evidenced

a fluid disomogeneous complex mass of 20 x 14 x 9 cm with fatty components and some hyperdense calcific areas (Figure 1). This mass dislocated the uterus to the right, the bowel loops to the upper-right quadrant and the bladder ahead. Hence the mass was presumed to have an ovarian origin. The serum tumor markers, human chorionic gonadotropin (β-hGC) and cancer antigen 125 (CA 125), assessed before the operation, were within normal limits, while alpha-fetoprotein (AFP) was high (5858 ng/mL; normal range 0-12 ng/mL) orienting to a diagnosis of ovarian YST.

An emergency laparotomy was performed; bloody ascites and peritoneal inflammation were found. No signs of macroscopic invasion of the pelvic structures were present. After the reduction of the cystic lesion by aspiration, a left salpingo-oophorectomy was performed (Figure 2).

Macroscopic anatomy showed an enlarged ovarian mass with large areas of necrosis and hemorrhage mixed with solid and cystic components. There was no evidence of left tube involvement. Microscopically, the tumor had an epithelial cell type appearance with Schiller-Duval bodies. On immunohistochemistry, tumor cells were positive for AFP and negative for CD30 and placental alkaline phosphatase (PLAP). Peritoneal washing cytology and multiple biopsies of the peritoneum and omentum were negative.

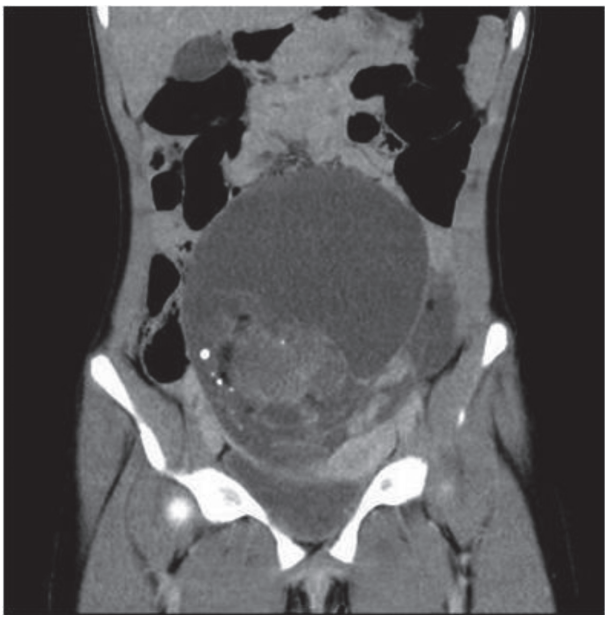

Figure 1. CT scan (sagittal section) showing a large pelvic mass, measuring 20 x 14 x 9 cm, with fatty components and some hyperdense calcific areas

Figure 2. Postoperative enlarged ovarian mass with large areas of necrosis and hemorrhage mixed with solid and cystic components

These findings were consistent with a diagnosis of YST associated to minor teratomatous lesions. The patient was discharged 5 days after the operation, and referred to the oncologist for a further treatment. An adjuvant chemotherapy regimen was performed according to the national comprehensive cancer network (4 cycles of bleomycin, etoposide and cisplatin: BEP) (7).Clinical surveillance did not reveal any chemotherapy-related toxicity.

A normalization of AFP level was observed within 2 months after surgery, suggesting an absence of residual tumor.

During 3 years of follow-up, the serum tumor markers and pelvic magnetic resonance imaging (MRI) showed no evidence of recurrence.

Discussion

A YST is the second most common malignant germ cell tumor and counts for only 1% of the malignant ovarian masses (8). It is most common in patients between 18 to 25 years. In most cases there are no clinical symptoms. However, abdominal swelling may be present in case of rapid growth. The size of tumors varies from 7 cm to 40 cm, with a median of 15-16 cm. The tumor is rarely bilateral (12-19%).

In symptomatic patients, abdominal pain is quite frequently complained leading to the discovery of the

disease, as seen in our patient. Clinical signs consist of an abdominal or pelvic mass, vaginal bleeding, fever, ascites or peritonitis secondary to torsion, infection or rupture of the ovarian tumor (9). The survival rate is higher in patients with earlier stage disease (stage I or II).

At present, the etiology of YST remains obscure. YST may develop from the false migration of embryonic primitive pluripotent germ cells or oncogenesis of the residual cells when they migrate from the genital ridge of the yolk sac endoderm (10).

These lesions typically determine abdominal pain with a rapidly enlarging abdominal mass (11). Moreover, like in this case, ascites or peritonitis, due to the torsion of the lesion, are rare additional features (12). CT scan and MRI are the most common techniques used to diagnose these lesions, but there are no specific signs to distinguish YST from other ovarian masses.

After treatment, periodic revaluations are required, such as abdominal and pelvic examination, CT scan, chest X-ray and AFP levels. YST are heterogeneous with a number of different histolopathological subtypes. The typical histopathological features of YST are solid, tubular and focal papillary patterns with Schiller-Duval bodies and sinusoidal structures with fibrovascular cores lining formed by tumor cells, frequent mitotic figures and are cytokeratin positive (13). In children, the vast majority of YST (85%) are in clinical stage I in comparison to a lower percentage (35%) in adults (14). AFP can be applied as a feasible tumor marker because its level is elevated in > 90% of YST (15, 16). In our case, the level of AFP was also increased (5858 ng/mL).

Therapeutic approach is based on surgery and chemotherapy, depending on the stage of the tumor based on FIGO classification. The aim of surgery is removing the primary ovarian tumor without excessive morbidity. The standard surgical staging consists of peritoneal washing, peritoneal biopsies, and biopsies of any suspicious lesions. Factors related to good prognosis are no ascites at presentation, stage I disease, less than 42 days to AFP normalization, fertility-sparing surgery and a serum AFP half-life less of 10 days (17).

The BEP is considered the gold-standard first-line treatment for germ cell tumors at all stages (18). Abdominal and pelvic examination, CT, chest X-ray and AFP levels are suggested during the follow-up (19). Progressive or recurrent ovarian tumour after treatment with BEP chemotherapy has been reported to be associated with a poor prognosis (17). The use of radiotherapy remains poorly defined because of its addiction to chemotherapy had no effect on improving survival.

During 3 years of follow-up, the serum tumor markers and pelvic MRI showed no evidence of recurrence.

Conclusions

YST are rare neoplasms that usually occurs in the first two decades of life. Surgery with adjuvant chemotherapy is the standard management. It is important for clinicians to remember that patients with YST can present abdominal pain in an unusual way. Serum AFP is a useful marker for the diagnosis and management of YST. More research is required for investigating the mechanisms of pathogenesis in order to develop more effective treatments for YST.

Ethics statement: We have obtained verbal informed consent from the patient's guardian for the publication of this case report.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

Symptomatic pseudoarthrosis secondary to a stress fracture of the acromion

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Summary. Fractures of the acromion are uncommon clinical entities, and stress fractures are even more rare, with few cases reported. Due to their rarity, stress fractures are often misdiagnosed. Here, we report a case of an elderly patient with an acromion stress fracture, which was overlooked, resulting in nonunion followed by the displacement of the distal portion of the acromion. The purpose of this report was to discuss this rare fracture, highlighting the importance of an accurate evaluation of radiological imaging as well as clinical data. (www.actabiomedica.it)

Key words: acromion, elderly patient, pseudoarthrosis, rotator cuff arthropathy, stress fracture

Introduction

Fractures of the acromion are uncommon (1) and mainly caused by significant trauma usually involving a direct downward force applied to the apex of the shoulder (2). Otherwise, they can result from an indirect force acting on the deltoid, or derive from overuse (3). Stress fractures of the acromion are even more rare with only few cases described in literature (2, 3). Most papers on stress injuries reported minimally displaced fractures diagnosed by radiographs and healed after a conservative treatment (3-7).

This report describes a case of a stress fracture of the acromion resulting in a nonunion in an elderly patient with a severe rotator cuff arthropathy.

Case Presentation

A 80 years-old right-handed lady came to our attention on March 2017 due to a 3-months history of severe pain and impairment on the right shoulder. Medical history included hypertensive cardiopathy, atrial fibrillation, and chronic kidney disease. She denied any previous and recent significant trauma, but reported a long history of pain and functional limitation due to a mild right rotator cuff arthropathy, managed by her family doctor with painkillers and physical therapy.

At the time of our examination, a mild swelling was observed above the acromion. Palpation of the acromial margins evoked acute pain. In addition, a hard and painful mobile body was palpable just few centimeters below the acromion. Passive mobilization of the shoulder was highly painful in all plans of movement, whereas active mobilization was seriously impaired. Standard radiographs revealed a substantial cranialization of the humeral head and a radiopaque body located laterally to the acromion described as a periarticular calcification (Figure 1a). A CT-scan was then performed (Figure 1b-c), showing a clear interruption of the distal portion of the acromion, with a free-flattened fragment located posterior-laterally. Since a displaced acromial fracture was suspected, previous radiological documentation of the patient was analyzed. The oldest available exams showed gleno-humeral and acro-

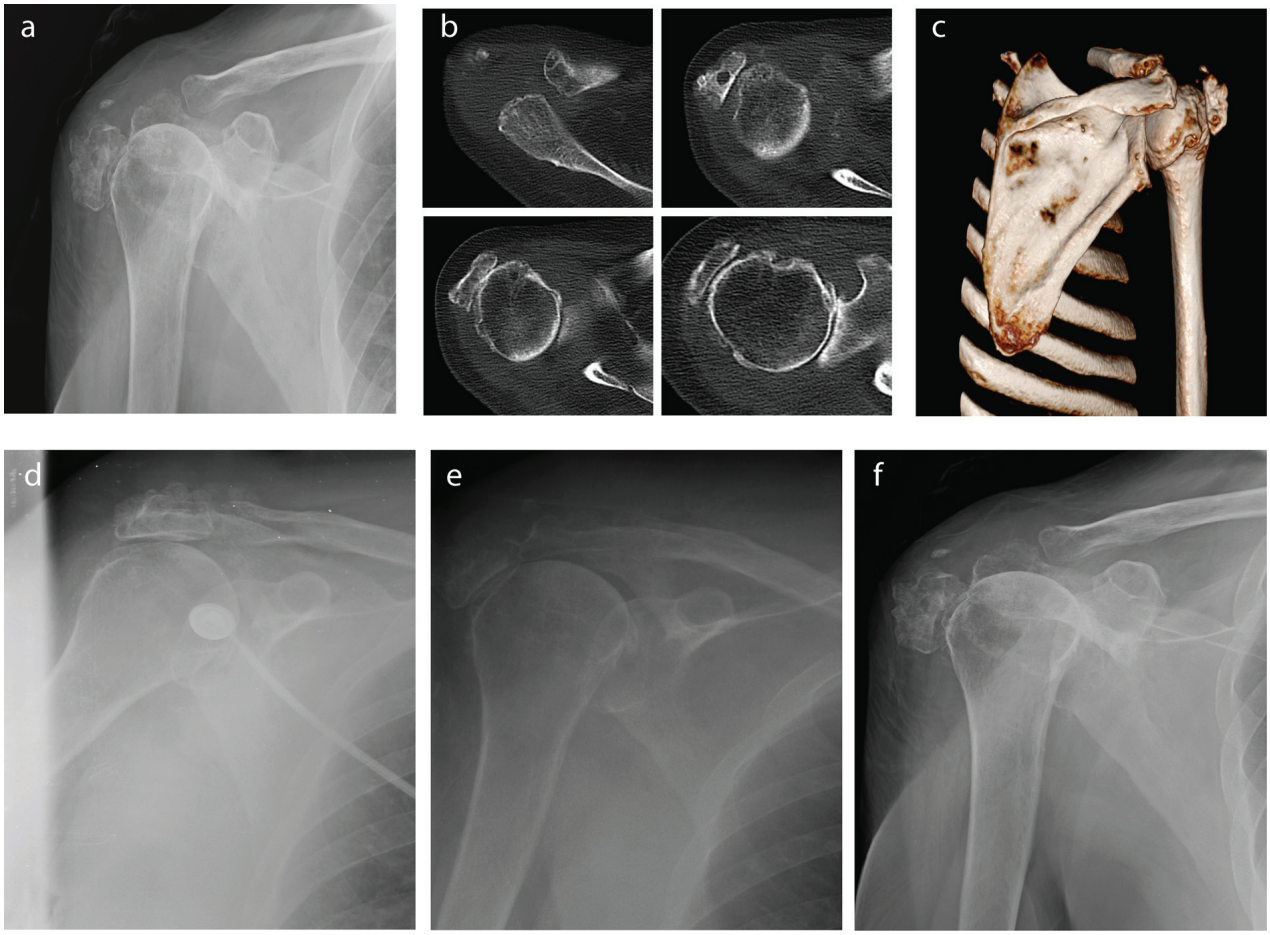

Figure 1. a) Radiographs of the shoulder show a periarticular calcified lesion; **b)** CT-scan of the shoulder, show a clear interruption of the distal extremity of the acromion and a free fragment located on the lateral aspect of the humeral head; **c)** 3D reconstruction of the CT-scan shows the posterior unusual aspect of the shoulder; **d-e-f)** progressive detachment of the acromial fragment during follow-up (Feb 2015, Dec 2016, Mar 2018, respectively)

mio-clavicular osteoarthritis, exclusively. On the other hand, chest radiographs performed two years (Figure 1d) and three months (Fig. 1e) before our visit, revealed an undisplaced fracture of the acromion and its subsequent lateral fragment detachment, respectively. Since the patient had not previous trauma, the lesion was recognized as a stress fracture of the acromion. Surgical fixation was proposed, considering fracture displacement and symptoms, but it could not be performed due to the poor patient's general conditions. At one year follow-up, the patient complained no pain at rest and slight pain during movements. She had a severe reduction in shoulder function: active extrarotation, flexion and abduction were particularly impaired, with a Constant score of 37/100 and a QuickDASH score of 45/55.

On palpation, the distal fragment of the acromion was still appreciable against the lateral aspect of the humeral head. At last follow-up, radiographs of the shoulder showed a partial reabsorption of the fragment (Fig. 1f), probably related to chronic friction.

Discussion

Several causes for acromial stress fractures have been described in literature: rotator cuff arthropathy (4, 5), history of reverse arthroplasty (8), manual lift-

ing work (3), carrying weight on the shoulder (9), and sport activities (2, 6, 7). As Dennis et al. and Roy et al. suggested, in the set of a rotator cuff arthropathy, the abnormal pressure of the humeral head on the acromion, could lead to acromial fracture (4, 10).

In the present case, despite symptoms were highly suggestive of an exacerbation of rotator cuff arthropathy, physical and radiological examination hinted a more complex lesion. The revision of the previous radiographs and their comparison with the clinical history enabled to reconstruct the pathogenesis of the lesion. Rotator cuff arthropathy led to a progressive cranial migration of the humeral head, causing prolonged subacromial friction and a consequent stress fracture of the acromion, which was facilitated by age-related osteoporosis. This occurred two years before our clinical check. Failure to recognize and treat the fracture, first led to the development of the nonunion and secondarily to the displacement of the distal fragment causing the sudden worsening of pain and loss of function characterized in the last 3 months.

This case represents a clear example of how these lesions can be easily overlooked, as a consequence of their rarity. A stress fracture should be suspected, and further studied, in patients complaining of sudden increase in rotator cuff-related pain. If the patient had been suitable for surgery, a reduction and fixation of the fragment should be considered to avoid the functional impairment of a nonunion. In conclusion, this rare condition should be kept in mind in absence of a proper resolution of symptoms.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Ethical approval: All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

Isolated superficial vein thrombosis in multiple thrombotic defects

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Summary. Superficial Vein Thrombosis (SVT) is generally a benign, self-limited disorder; however, when larger veins are involved, propagation into deep vein system can occur. Thrombophilic disorders denote several risk factors that predispose individuals to develop venous thromboembolism. It includes inherited thrombophilia, acquired thrombophilia and hyperhomocysteinemia. For thrombophilic patients who develop SVT, the current guidelines don't clarify for how long time anticoagulation therapy must be performed. We describe a case of SVT in a patient with multiple thrombotic defects, for which we starting Fondaparinux 2.5 mg/die and stopped it when the SVT was solved. During the follow-up period (eighteen months) no Deep Vein Thrombosis or Superficial Vein Thrombosis were detected. Clinical surveillance seems to have been the best strategy for this patient. We believe that this single case report emphasizes the needed of prospective studies to verify the risk of thromboembolic recurrence in thrombophilic patients with SVT. (www.actabiomedica.it)

Key words: anticoagulation, low molecular weight heparin, phlebitis, thrombophilia, ultrasound, symptomatic, risk factors

Abbreviation list
SVT=Superficial Vein Thrombosis
APS=Antiphospholipid Syndrome
VTE=Venous Thromboembolism
aPLs=Antiphospholipid Antibodies
DVT=Deep Vein Thrombosis
aCL=Anticardiolipin
B2GPI=beta 2-glycoprotein I
LAC=Lupus Anti Coagulant
Ig=Immunoglobulin
PE=Pulmonary Emobolism

Background

Superficial Vein Thrombosis (SVT) is generally a benign, self-limited disorder; however, when larger veins are involved, propagation into deep vein system, as Deep Vein Thrombosis (DVT) and even Pulmonary Embolism (PE) can occur (1). Treatment is aimed at relieving local symptoms and preventing thromboembolic complications. SVT has been less well studied than DVT but is estimated to occur more often (2, 3). It usually affects lower limbs, often involves varicose veins. Can be unprovoked or associated with several conditions such as malignancy, chronic venous insufficiency, pregnancy or estrogen therapy, sclerotherapy, obesity, history of VTE, long-distance travel, thrombophilia (2-4).

Thrombophilic disorders denote several risk factors that predispose individuals to develop venous thromboembolism. It includes inherited thrombophilia, acquired thrombophilia and hyperhomocysteinemia.

Regarding the inherited form, mutation of Factor V gene is the most common (more often in the heterozygous form), while the prothrombin gene mutation, protein S, protein C, and antithrombin deficiencies account for most of the remaining cases.

The most common acquired form of thrombophilia is the antiphospholipid syndrome (APS). APS

is characterized by venous or arterial thrombosis and/or pregnancy morbidity in the presence of persistent laboratory evidence of antiphospholipid antibodies (aPLs) (5). In APS patients can present with both venous and arterial thrombosis.

Severe hyperhomocysteinemia is generally due to a rare autosomal recessive disorder characterized by severe elevations in plasma and urine homocysteine concentrations. It is related to MHTFR gene mutation in homo or heterozygosis. There is increasing evidence that hyperhomocysteinemia is a risk factor for VTE (6).

For thrombophilic patients who develop SVT, the current guidelines don't clarify for how long time anticoagulation therapy must be performed.

Case Report

A 48 years-old woman was admitted to our ward for erythema and swelling of the left lower limb, suspected of DVT. We perform thrombophilic screening and a venous ultrasound of the lower limbs.

Thrombophilic screening highlighting heterozygous mutation for the Factor V gene, the homozygous mutation for MTHFR C677T gene, the increase of anti-beta 2-glycoprotein I (B2GPI) antibody IgM and anti-cardiolipin (aCL) antibody IgM. Remaining tests were in the normal range (Table S1 summarize all blood test performed). The venous ultrasound doesn't highlight any acute thrombotic events. We don't start any anticoagulation therapy.

As current guidelines recommended, the laboratory tests for aPLs were repeated at 12 weeks. A downward trend was observed (Figure 1, Table 2).

Table 1. Blood tests performed at the first clinical observation

Blood tests performed	Patient values	Normal values
WBC	5,91	4000-10000/mm3
RBC	3,57	3900000-5200000/mm3
HB	11,7	12-16 g/dl
HT	34,5	36-46%
PLT	178000	150-400000/mm3
PT INR	0,94	0.86-1.14
aPTT ratio	1,01	0.82-1.18
ATIII	124	>83%
C-Protein	106	>70%
S-Protein	85	>60%
aCL IgM	45,5	< 10 MPL-U/ml
aCL IgG	2,42	< 10 GPL-U/ml
B2GPI IgM	59,6	<7 U/ml
B2GPI IgG	1,76	<7 U/ml
LAC	0,97	<1.2 ratio
Leiden's Factor	Heterozygous mutation	Omozygous normal
Factor II	Omozygous normal	Omozygous normal
Homocysteinemia	12,5	<15 micromol/L

WBC=White Blood Cell; RBC=Red Blood Cell; HB=Hemoglobin; HT=Hematocrit; PLT=Platelets; PT=Prothrombin Time; aPTT=activated Partial Thromboplastin Time; INR=International Normalized Ratio; LAC=Lupus Anti Coagulant; aCL=Anticardiolipin; B2GPI=beta 2-glycoprotein I; ATIII=Antithrombin III; Ig=immunoglobulin

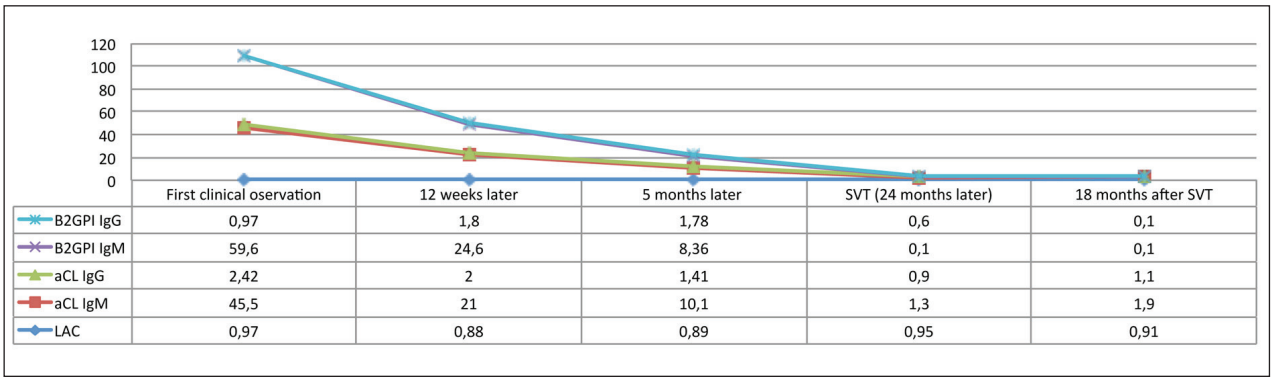

Figure 1. Trend of laboratory antiphospholipid antibodies; B2GPI=beta2 glycoprotein I; aCL Anticardiolipin antibody; LAC=Lupus AntiCoagulant antibody

After 5 months patient repeated aPLs blood test: AB2GPI IgM and aCL IgM were decreasing but aCL IgM was still over the normal range (Figure 1, Table 3).

Two years later, patient cames again at our attention for swelling of the left lower limb. A venous ultrasound detects Superficial Vein Thrombosis (SVT) of Leonardo's vein (collateral posterior vein of Great Safenian). No travel, no immobilization or any risk's conditions are present in the previous days. Patient's didn't suffer from varicose veins. Then, SVT has been baptized as "idiopathic". Fondaparinux 2.5 mg/die was started and after 30 days of treatment SVT was completely regressed and Fondaparinux stopped. A new thrombophilic screening was sent at the time of SVT, but the results weren't available when Fondaparinux was stopped. Only 15 days after, we discovered that aPLs were in the normal range at the time of SVT (Figure 1, Table 4).

Eighteen months after SVT, patient cames for follow up visit. We have repeated laboratory aPLs screening and venous ultrasound of the lower limbs. No DVT or SVT were detected. aCL and B2GPI were again in the normal range (Figure 1, Table 5).

Table 2. Blood tests performed 12 weeks later

Blood tests performed	Patient values	Normal values
aCL IgM	21	<10 MPL-U/ml
aCL IgG	2	<10 GPL-U/ml
B2GPI IgM	24,6	<7 U/ml
B2GPI IgG	1,8	<7 U/ml
LAC	0,88	<1.2 ratio

aCL=Anticardiolipin; B2GPI=beta 2-glycoprotein I; LAC=Lupus Anti Coagulant; Ig=immunoglobulin

Table 3. Blood tests performed 5 months later

Blood tests performed	Patient values	Normal values
aCL IgM	10,1	<10 MPL-U/ml
aCL IgG	1,41	<10 GPL-U/ml
B2GPI IgM	8,36	<7 U/ml
B2GPI IgG	1,78	<7 U/ml
LAC	0,89	<1.2 ratio

aCL=Anticardiolipin; B2GPI=beta 2-glycoprotein I; LAC=Lupus Anti Coagulant; Ig=immunoglobulin

Table 4. Blood tests performed during Superficial Vein Thrombosis

Blood tests performed	Patient values	Normal values
aCL IgM	1,3	<10 MPL-U/ml
aCL IgG	0,9	<10 GPL-U/ml
B2GPI IgM	0,1	<7 U/ml
B2GPI IgG	0,6	<7 U/ml
LAC	0,95	<1.2 ratio

aCL=Anticardiolipin; B2GPI=beta 2-glycoprotein I; LAC=Lupus Anti Coagulant; Ig=immunoglobulin

Table 5. Blood tests performed 18 months after SVT

Blood tests performed	Patient values	Normal values
aCL IgM	1,9	<10 MPL-U/ml
aCL IgG	1,1	<10 GPL-U/ml
B2GPI IgM	0,1	<7 U/ml
B2GPI IgG	0,1	<7 U/ml
LAC	0,91	<1.2 ratio

aCL=Anticardiolipin; B2GPI=beta 2-glycoprotein I; LAC=Lupus Anti Coagulant; Ig=immunoglobulin

Discussion and review of the litterature

The patient's thrombotic pattern known at the time of SVT was: a positivity of aPLS (although in decreasing, considering the last laboratory test performing 2 years before), heterozygous mutation of Factor V gene, Homozygous mutation of MTHFR C677T gene. The above-mentioned gene's mutations don't identify a reason for long-term anticoagulation, although in SVT or DVT. We wondered if aPLs increase could have a role in the long-term therapeutic choice when we decide to stopped Fondaparinux.

The clinical criteria to diagnose APS (7) include objectively confirmed venous (DVT or Pulmonary Embolism), arterial or pregnancy complications associated with a positive laboratory test for 1 or more aPLs found on 2 or more occasions at least 12 weeks apart. The aPLs recognized in the international criteria include 1) lupus anticoagulant (LAC) detected according to guidelines published by the International Society on Thrombosis and Haemostasis (ISTH), (8), 2) anticardiolipin (aCL) antibody (IgG or IgM) exceeding 40 IgG or IgM phospholipid units, 3) anti-

beta2GPI antibody (IgG or IgM) at titers exceeding the 99th percentile.

In the patient of our case report, aPLs abnormality was confirmed at 12 weeks and at 5 months, although in decreasing.

In APS long life anticoagulation therapy using Warfarin or Acenocoumarol is recommended, while the use of Direct Anticoagulants (DOACs) are, at time, debated (9, 10).

When Fondaparinux was stopped, we had to take a difficult therapeutic choice considering patient's anamnestic positivity for aPLs.

Can isolated SVT change the long-term risk profile in patients with aPLs without VTE? Can SVT be compared to PE or DVT in this group of patients? Zuilly et al. performed a prospective cohort study on 92 patients without anticoagulant treatment (follow up period 35 months) to determine risk factors for thrombotic events in patients with aPLs and/or systemic lupus erythematosus. In multivariate analysis, SVT (HR, 7.45 [95% confidence interval, 2.25 to 24.66]) was an independent risk factor for thrombotic events (11).

The authors highlighting an increased risk of VTE in patients with SVT and thrombotic defect, but don't clarify whether long-life anticoagulation is justified in this patients.

The guidelines of the British Committee for Standards in Haematology recommend that confirmed SVT should be considered for therapeutic anticoagulation, although the optimal duration is uncertain (12).

For the patient of this case report, we decided to stop Fondaparinux when SVT was solved. We don't start long-term anticoagulation or prophylaxis, preferring the clinical surveillance. Our reasons were: first, although SVT shares some risk factors with DVT, it doesn't define APS when associated with aPLs. The anticoagulation-related long-term bleeding risk is not justified in SVT although occur in thrombophilic patients. Second, prospective studies on the long-term risk of thromboembolic recurrence in this subgroup of patients have not been performed. This risk is, at time, unknown. Third, aPLs at time of diagnosis was over the normal range, but in decreasing. We have preferred to check again aPLs in order to verify the auto-antibodies trend. Finally, the homozygous mutation of MTHFR C677T gene and the heterozygous mutation of Factor V gene, the latter common in the general population with the prevalence of 3-5% (13), doesn't justify long-term anticoagulation in case of DVT or SVT. Appropriate antithrombotic prophylaxis in all high thrombotic risk conditions (such as pregnancy, long-distance travel, immobilization for any causes, surgery) is enough in this subgroup of patients.

Conclusion

We believe that this single case report emphasizes the needed of prospective studies to verify the risk of thromboembolic recurrence in thrombophilic patients with SVT. For the patient of this case report, clinical surveillance seems to have been the best strategy.

Disclosure of Conflict of Interests:
Dr. Carmine Siniscalchi received speaker's fee for congress presentation by MediK.

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Care for others: the female perspective

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In 1792, Mary Wollstonecraft (1759-1797) claimed women's inclusion in public life and published the famous "A Vindication of the Rights of Women", considered one of the earliest works of the English feminist movement (1). She argued that every man has an equal right to education because of his equal intrinsic capability to reason. In response to this book, in the same year in London, the philosopher and translator Thomas Taylor (1758-1835) wrote, anonymously, a satire provocatively entitled "A Vindication of the Rights of Brutes" (2).

In his writing, the Neoplatonic philosopher of Cambridge explicitly criticized the thesis of the British writer: "If one accepts that all men have equal rights, one also has to accept that all women have rights, which unfortunately leads to the conclusion that all brutes have rights, which proves that the initial argument about the rights of men must be wrong".

Taylor's words, however, should not amaze us because for thousands of years of history and Jewish-Christian tradition, philosophical thought has brought together women, children, slaves and animals "in the same condition of subjection with respect to man, white, western and hunter".

However, fate is mocking us since the knowledge of the satirical libel is today linked to the moral reasonableness of the thesis of recognizing rights to non-human animals, at that time absurd, as that of the recognition of rights for women. Moreover, the woman-animal binomial proposed by Taylor has, albeit unconsciously, favored the link between the animal question and feminism expressed today in the ecofeminism movement.

Women at the time were not considered to be totally rational beings and female instability was empirically founded by the presence of the uterus that forced women to the mere rules of corporeity. Rationality, as a masculine attribute, justified the state of superiority of man over woman and, likewise, over the animal world.

Probably, the fear that the emotional-animalistic approach could undermine the female emancipationist cause has delayed the interest of the female movement towards the rights of non-humans, unlike what happened to other battles, such as anti-racism, anti-fascism anti homophobia and transphobia or class struggle. Women claimed their equality with men, on the basis of the equality of their rational faculties. Approaching the struggle of women's rights to that of animals could be considered a sore point capable of undermining the validity of the claimants theses.

Indeed, after a long period of time, medicine abandoned certain scientific ideas about the natural inequalities and the mental inferiority of women compared to men.

For centuries, access to study and medical careers for women have been severely opposed.

Recent researches indicate that women tend to be more concerned about the welfare of humans, animals and the natural environment more than men (3,4). Perhaps it is curious to point out that a psychiatric illness such as autism, based on the total lack of empa-

thy, is much more widespread among men than among women (5).

It is essential to note that the presence of empathy, far from representing an element of weakness and fragility, is a powerful communication skill that has positive benefits for both clinician and patient: increases the efficiency of gathering information, and honours the patient.

Therefore, it is worth remembering the words that a sick friend gave Elizabeth Blackwell, the first woman in modern history to graduate in medicine and practice this profession in the United States of America:

"You are fond of study, have health and leisure; why not study medicine? If I could have been treated by a lady doctor, my worst sufferings would have been spared"(6)

On the basis of empathy and the ties it establishes, animal assistance interventions are increasingly valued in various healthcare environments to promote well-being, social integration and communication.

Animals and women are united because they continue to suffer increasing violence and oppression yet, in both cases, they are underestimated. Animals and women united in the claim of the fundamental duty to respect, indispensable for making the behavior of man ever more empathetic towards the other, the suffering, the different, the foreigner, the disadvantaged, the non-human (7, 8).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Metabolic alkalosis in patients with distal renal tubular acidosis

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To the Editors:

I read with great interest the article in your journal by Lucaccioni et al. entitled "Severe metabolic alkalosis due to diuretic treatment in a patient with distal renal tubular acidosis: a rare association" (1). The authors reported a neonate with distal renal tubular acidosis (dRTA) who presented with hyperchloremic metabolic alkalosis after hydrochlorothiazide (HCT) treatment.

Although extremely rare, 2 infants with dRTA who developed metabolic alkalosis have previously been reported (2, 3). McSherry reported a 4-month-old infant with dRTA who developed hypochloremic metabolic alkalosis and severe hypokalemia following HCT treatment (2). Hymes et al reported a 7-month-old infant with dRTA who presented with hypochloremic metabolic alkalosis and hypokalemia after a 4-day history of vomiting, without HCT treatment (3). The authors of both reports suggested that severe metabolic alkalosis in their patients appeared related to hypokalemia and extracellular volume depletion.

Metabolic alkalosis can be caused by potassium depletion and/or chloride depletion due to gastric fluid loss or the chloruretic effect of a diuretic (4). The patient in the report by Lucaccioni et al. presented with hypokalemia, hypochloremia, and dehydration following HCT treatment and vomiting, leading to severe metabolic alkalosis (1). Patients with dRTA who have hypokalemia and are treated by a thiazide diuretic and/or have persistent vomiting episodes should be need to be caution for the onset of metabolic alkalosis.

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Original article

Improved efficiency and cost reduction in the emergency department by replacing contemporary sensitive with high-sensitivity cardiac troponin immunoassay

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Summary. *Background:* Although unquestionable evidence has been provided that high-sensitive (HS) cardiac troponin (cTn) immunoassay outperform the former contemporary-sensitive techniques, some clinicians are still hesitant to implement HS methods in routine clinical practice. This study was hence planned to evaluate the impact of replacing a contemporary-sensitive with HS cTnI immunoassay on hospital and laboratory workload. *Methods:* Information on the total number of cTnI tests ordered, total number of blood samples collected, total number of CK-MB tests ordered, number of patients with the first HS-cTnI value below the limit of detection (LoD) and cumulative HS-cTnI values was extracted from the local hospital information system for the semesters before and after the HS method was introduced. *Results:* Although the total emergency department (ED) visits modestly increased after introducing HS-cTnI, the number of total cTnI tests declined by over 10%. A substantial reduction of single-sample test requests was noted, accompanied by a considerable decline of 3- and 4-sample collections (i.e., -61% and -73%, respectively). A high percentage of patients (27.5%) displayed HS-cTnI values <LoD at admission, thus allowing safe discharge. The introduction of HS cTnI immunoassay was effective to collapse the number of CK-MB test requested and also generated favorable return of investment for the part of laboratory budget concerning cTnI testing (-3.2% of total costs for cTnI and -99.8% of total costs for CK-MB). *Conclusion:* The results of this study show substantial organizational and economic benefits by replacing contemporary-sensitive with HS cTnI immunoassays. (www.actabiomedica.it)

Key words: cardiac troponin, high-sensitivity, myocardial infarction, acute coronary syndrome, emergency department

Introduction

The development and introduction into clinical practice of high-sensitivity (HS)-cardiac troponin (cTn) immunoassays have consistently modified the diagnostic approach to patients with suspected acute coronary syndrome (ACS) during the last two decades (1). These novel immunoassays have remarkably amplified the diagnostic performance of cardiac biomarkers testing (especially in patients with non-ST elevation myocardial infarction; NSTEMI) and can also generate important clinical evidence of cardiac injury be-

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yond myocardial ischemia. Supported by reliable data (2-4), the fourth universal definition of myocardial infarction has endorsed and disseminated these concepts (5), so that the usage of HS-cTn immunoassays shall be considered virtually unavoidable now. Despite this evidence, some clinicians are still hesitant to replace the former so-called contemporary-sensitive methods with HS-cTn techniques, justifying this reluctance with concerns of overutilization, possible overdiagnosis of cardiac injuries, overcrowding of emergency departments (EDs), and excess of cardiac invasive testing. This translates into the reliable evidence that "contemporary-sensitive" or conventional (i.e., non-HS) cTn immunoassays, as well as myoglobin and creatine kinase MB (CK-MB) tests, are still largely used around the world (6-8).

With some differences due to the analytical characteristics of the assay, the hospital organization, and the clinicians' background, the most common approach for diagnosing ischemic and non-ischemic myocardial injury encompasses a baseline measurement of cardiac biomarkers followed, when needed (i.e., values not diagnostic at the first measurement or non-diagnostic electrocardiogram), by serial sampling at different times points in order to detect suggestive changes in cTn and distinguishing thus an acute from a chronic cardiac damage (9, 10). Importantly, whether the "absolute delta" (i.e., the absolute variation of cTn troponin value between two time points), the "relative delta" approach (i.e., the percentage variation of cTn troponin value between two time points) or even an "integrated" approach (i.e., combining both modalities of calculating cTn variation, depending on the baseline value) would provide better diagnostic performance remains to be precisely defined (11).

In this study, we aimed to investigate the impact on hospital and laboratory workload consequent to the shift from a contemporary-sensitive toward a HS-cTnI immunoassay. For this purpose, we retrospectively analyzed data from two separate semesters in the local hospital, the first when a conventional cTnI assay has been used and the second few months after routine introduction of a HS-cTnI assay.

Materials and Methods

On April 16th, 2018, the University Hospital of Parma shifted from a contemporary-sensitive cTnI technique (Accu-TnI+3 -A98264. Beckman Coulter; Brea CA) toward a HS immunoassay (Access-HsTnI-B52700. Beckman Coulter; Brea CA). The specific characteristics of these assays have been previously described elsewhere (12, 13). Briefly, the values of the limit of blank (LoB), limit of detection (LoD) and 99th percentile of the upper reference limit (URL) of the former and new HS-cTnI immunoassays were 9 ng/L, 13 ng/L and 34 ng/L for AccuTnI+3 and 0.14 ng/L, 0.34 ng/L and 17.8 ng/L in men and 10.5 ng/L in women for Access-HsTnI, respectively. Despite the test exhibits much better analytical performance, the cut-off of 2.3 ng/L for LoD of the HS-cTnI was locally used for rule-out, as currently suggested by the manufacturer. After the introduction of Access HsTnI, CK-MB was contextually eliminated from the local "cardiac entry panel" so that its prescription remained only available at clinicians' discretion. Several meetings, involving emergency physicians (EPs), cardiologists, laboratory professionals, and other hospital specialists, were organized in the three months before changing the cTnI immunoassay aimed at disseminating the knowledge on clinical use and interpretation of HS-cTnI.

The EPs were free to decide about timing for HS-cTnI sampling according to their clinical judgment, mostly based on available guidelines (14). Before the shift to HS-cTnI, blood sampling had been performed at 0, 3 and 6 hours. Although very rarely, further samples could still be collected when clinical suspicion remained high even after 6 hours from patient admission. This previous timing for sample collection was then maintained after introducing the HS-cTnI immunoassay. Nevertheless, in accordance with current guidelines (5), the EPs were instructed that in the vast majority of cases the 2nd sample (i.e., collected 3 hours from admission) shall be considered "diagnostic" (i.e., allowing safe diagnosis or rule out of AMI), thus making additional (later) testing almost unnecessary.

The months closer to the shift from the contemporary-sensitive to the HS-cTnI immunoassay were excluded from statistical analysis in order to allow a

sufficient period of customization with the new test. Therefore, all records of patients visited in the ED of the University Hospital of Parma for whom at least one cTn test had been ordered between October 1st, 2017 and March 31st, 2018 (i.e., the semester before the immunoassay was changed), and between October 1st, 2018 and March 31st, 2019 (the semester after the immunoassay was changed) were extracted from the local hospital information system (LIS). The following data were analyzed throughout the study periods: (a) total number of cTnI tests ordered; (b) total number of blood samples collected; (c) total number of CK-MB tests ordered; (d) number of patients with the first HS-cTnI value below the limit of detection (LoD) who have been ruled out without additional tests, and (e) number of patients exhibiting either HS-cTnI values below LoD or comprised between the LoD and the URL, for whom a second HS-cTnI sampling was requested.

The statistical analysis was then carried out with Analyse-it (Analyse-it Software Ltd, Leeds, UK) and MedCalc statistical software (MedCalc Software, Ostend, Belgium). Due to the retrospective nature of the study and the maintenance of anonymity of all subjects, the consensus of the ethical committee was unnecessary. The study was performed in accordance with the Declaration of Helsinki and under the terms of relevant local legislation.

Results

The total number of ED visits during the two study periods is summarized in table 1. Although a modest increase of total ED visits occurred between the two semesters when contemporary-sensitive (first semester) or HS-cTnI (second semester) were used (+ 659; +1.1%), a considerable decrease were recorded in the total number of cTnI tests during the second semester (-779; -7.6%). This decline was even more evident when data were normalized for the number of total ED visits (see Table 2). Overall, the relative decrease of cTnI tests was 10% after introduction of HS-cTnI immunoassay in the second semester ($p<0.001$).

The number of samples collected from each ED patient during the two different semesters (before and after introducing HS-cTnI), is shown in table 3. A substantial reduction of single-sample test requests was noted, accompanied by a considerable decline of 3- and 4-sample collections (i.e., -61% and -73%, respectively), thus reflecting a higher number of patients who could be ruled out with only two samplings in the semester after the HS-cTnI immunoassay was implemented. Out of the 6281 patients with a single HS-cTnI value, 1731 displayed a value <LoD at admission (27.5%), which together with low clinical risk would have permitted safely discharge, thus significantly contributing to reducing overcrowding in the ED. The val-

Table 1. Number of emergency department (ED) visits and cardiac troponin I (cTnI) tests during the two-study period, before (October 217 to March 2018) and after (October 2018 to March 2019) introducing the high-sensitivity (HS) technique

Period	ED visits			cTnI tests in the ED		
	Before HS-cTnI	After HS-cTnI	Variation	Before HS-cTnI	After HS-cTnI	Variation
Year	**2017**	**2018**		**2017**	**2018**	
October	10112	10017	-0.4%	1642	1495	-8.9%
November	9356	9204	-1.6%	1625	1535	-5.7%
December	10020	9654	-3.6%	1730	1576	-9.0%
Year	**2018**	**2019**		**2018**	**2019**	
January	10120	10122	=	1861	1753	-6.0%
February	9011	9685	+7.5%	1548	1568	+1.2%
March	9750	10346	+6.1%	1825	1525	-16.5%
Total semester	58369	59028	+1.1%	10231	9452	-7.6%

HS-cTnI, high-sensitivity cardiac troponin I

Table 2. Percentage (%) of cardiac troponin requests on total emergency department (ED) visits before (October 217 to March 2018) and after (October 2018 to March 2019) introducing the high-sensitivity (HS) technique

Period	Percentage (%) of cardiac troponin requests on total ED visits		Odds ratio (95% CI)
	Before HS-cTnI	After HS-cTnI	
Year	**2017**	**2018**	0.90 (95% CI, 0.84-0.98; p=0.010)
October	16.2	14.9	0.95 (95% CI, 0.88-1.03; p=0.210)
November	17.4	16.7	0.93 (95% CI, 0.87-1.01; p=0.078)
December	17.2	16.3	0.93 (95% CI, 0.87-0.99; p=0.047)
Year	**2018**	**2019**	
January	18.4	17.3	0.93 (95% CI, 0.86-1.01; p=0.070)
February	17.2	16.2	0.75 (95% CI, 0.67-0.81; p<0.001)
March	18.7	14.7	0.90 (95% CI, 0.84-0.98; p=0.010)
Total semester	17.5	16.0	0.90 (95% CI, 0.87-0.93; p<0.001)

HS-cTnI, high-sensitivity cardiac troponin I

Table 3. Number of blood sampling in emergency department patients in whom at least one cardiac troponin I (cTnI) test was requested

	Before HS-cTnI		After HS-cTnI		Odds Ratio (95% CI)
	N.	%	N.	%	
Only one sample	7572	74.0	6381	67.5	0.73 (95% CI, 0.69-0.78; p<0.001)
Two samples	1282	12.5	2562	27.1	2.60 (95% CI, 2.41-2.80; p<0.001)
Three samples	1210	11.8	467	4.9	0.39 (95% CI; 0.35-0.43; p<0.001)
Four samples	167	1.6	42	0.4	0.27 (95% CI, 0.19-0.38; p<0.001)
Total	10231	100	9452	100	-

HS-cTnI, high-sensitivity cardiac troponin I

Table 4. Distribution of high-sensitivity cardiac troponin I (HS-cTnI) values in emergency department patients in whom a second blood sample was requested

HS-cTnI value	Number	%
<LoD	403	15.7%
>LoD and <URL	1229	48.0%
- In women	- 460	- 17.9%
- In men	- 769	- 30.1%
>URL	930	36%
Total	2562	100%

LoD, limit of detection; URL, 99th percentile of the upper reference limit; HS-cTnI, high-sensitivity cardiac troponin I

ue of HS-cTnI in patients in whom a second test was requested is shown in table 4. Overall, 36% of these patients displayed a HS-cTnI value >URL, whilst 48% exhibited a value comprised between LOD and URL (17.9% women, 30.1% men) and 15.7% <LOD, respectively.

Despite the new HS-cTnI assay has a cost per test slightly higher than that of the former contemporary sensitive technique (1.02 versus 0.96€, based on the local tender), the lower number of tests led to a significant reduction in the cumulative cost of cTnI assessment in the local ED (from 9821 to 9603€; -2.2%), which decreased by further 100€ considering also the lower number of tubes collected after introducing the

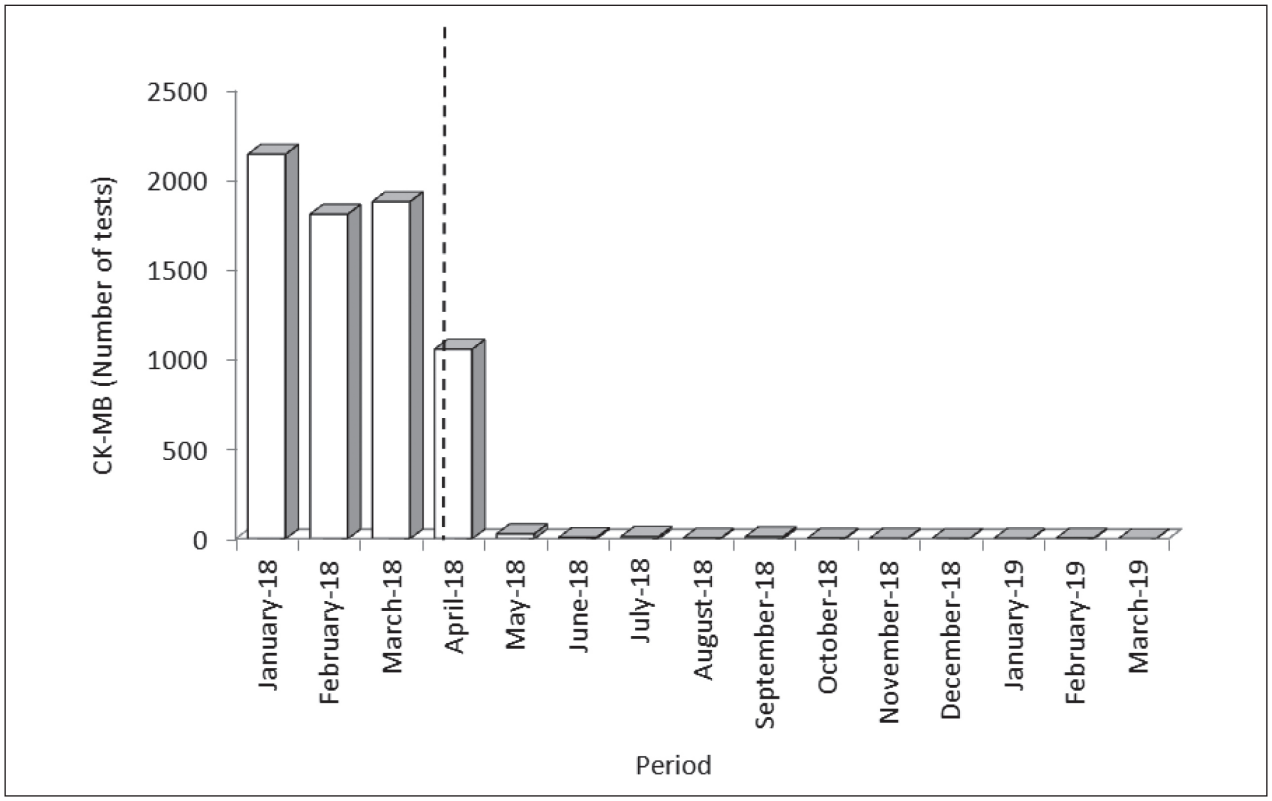

Figure 1. Graphical representation of the drop in creatine kinase MB (CK-MB) requests from the emergency department after shifting toward HS-cTnI. The dotted line defines the day of introduction of HS-cTnI immunoassay.
CK-MB, creatine kinase MB

HS-cTnI immunoassay (i.e., final estimated saving, -3.2%). The trend of CK-MB test requests made in the ED is summarized in figure 1. A collapse can be observed in the overall number of requests, then stabilizing around 2 requests per month (between October 2018 and March 2019). In the period limited to the trimesters before and after the introduction of HS-cTnI, the overall number of requests for CK-MB declined by over 90% (OR, 0.098; 95% CI, 0.091-0.106; p<0.001). From an economic point of view, these results entail remarkable savings since the overall budget for CK-MB can be estimated to decrease from 23811 to 40€ per year (-99.8%) in the local laboratory.

Discussion

Although the HS-cTn immunoassay have only been developed around 10 years ago, and despite an overwhelming burden of recent data, several clinicians are still reluctant to accept the evidence supporting the analytical and clinical advantages of using HS-cTn immunoassays and remain hesitant to implement these methods into clinical pathways (15). Several factors have lead clinicians to use terms such as "troponin leak", "false-positive" troponin elevation, or "troponinemia". These terms are trivial and reflect only evidence of confusion and "fear of the unknown" so that they shall be avoided (16, 17).

In agreement with previous evidence (18-20), the results of our study confirm that, in a real-world setting, the better diagnostic accuracy of HS-cTnI may also improve ED efficiency and decrease the overall costs, which is an additional aspect in favor of introducing HS techniques for more timely and accurate management of patients admitted to the ED with symptoms suggestive of ACS. Another remarkable advantage was the possibility to persuade the local clinicians that us-

ing a HS-cTnI immunoassay, then CK-MB could be considered redundant or obsolete and could be safely abandoned. This paradigm shift has been unsuccessfully attempted many times before, when the contemporary-sensitive immunoassay was still in use (21), although it could only be concretized after introducing the HS method and the relative guidelines (5). Notably, the introduction of the HS cTnI immunoassay not only was effective to collapse the number of CK-MB test requests but also generated a favorable return on investment for cTnI testing. However, additional efforts shall be promoted among EPs to operate following the current guidelines for HS-cTnI testing and for harmonizing the practices, whereby the number of potentially inappropriate second blood sample collections remained extremely high (i.e., ~64%) even after introducing the HS-cTnI immunoassay.

Considering that the ED environment is worldwide increasingly overcrowded, further studies on a real-world clinical, economic, and organizational impact of the introduction of the new HS-cTn assays should be prioritized to give clinicians the best tools to rule out patients presenting to EDs with suspicion of ACS. This fact would help EPs to avoid that the increasing burden of ACS around the world would be translated into a "perfect storm" in the ED (22).

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Original article

Workplace violence against healthcare workers in Emergency Departments. A case-control study

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Summary. *Background and aim:* Assessment and management of workplace violence (WPV) towards healthcare workers (HCWs) employed in Emergency Departments (EDs) represents a challenge for healthcare organizations worldwide. To date there is a lack of scientific data about the impact of work-shifts on the occurrence of WPV against ED HCWs. The purpose of this study was to investigate the relationship between work shift schedules and WPV against registered nurses (RNs) working on non-traditional shifts, including nights and 12-hour shifts. *Methods:* The authors conducted a cross-sectional nested case-control analysis of data regarding the episodes of WPV perpetrated by patients or their relatives against RNs employed in three EDs, in the period between January –December 2017. *Results:* The one-year incidence of WPV was 29,30 per 100 Full Time Equivalent (FTE) positions. Cumulative nightshifts were significant for 3 or more nightshifts compared to working less than 3 nightshifts during the 7 days before the episodes of WPV; additionally, RNs working 9 or more night-shifts showed higher risk of experiencing WPV compared to RNs working less than 4 night-shifts in the previous 28 days. *Conclusion:* In the present study shift work and WPV occurrence against ED RNs resulted interconnected; improvement interventions aimed at preventing the WPV should consider the characteristics of work shift schedules with the purposes of: 1) limiting the night shifts up to two per week and up eight per month; 2) adopting constant forward-rotating shift schedules. (www.actabiomedica.it)

Key words: assault, risk assessment, risk management, nurse, shift work

Introduction

Globally, healthcare organizations face the concern of workplace violence (WPV) perpetrated by patients against healthcare workers (HCWs) employed in Emergency Departments (EDs). In fact, by literature, between 24% and 88,8% of ED HCWs have been victim of violence by a patient in a 12-month period (1-3); this large range of occurrence is due to: 1) non-unique definition of workplace violence adopted by the authors in their studies (i.e. some studies analyzed only physical assaults and not considered verbal violence or sexual harassment); 2) phenomenon of under-reporting of WPV by the victims, due to many factors (i.e. fear of retaliation from aggressor and his/her family, feelings of shame related to being the subject of aggression, or addiction to WPV considered an integral part of job) (4). In many studies the predictors of violence perpetrated by patients against HCWs turned out to be linked with patients' diseases: dementia, schizophrenia, anxiety, acute stress reaction, suicidal ideation, and alcohol and drug intoxication are frequently related to WPV (5, 6). A growing literature evidenced the role of issues within the EDs in causing the occurrence of WPV. Particularly, inadequate HCW-patient relationship, high anxiety level among the staff, poorer perceived safety climates, high job demands, have been demonstrated as strongly and positively associated to WPV occurrence (7, 8). In a recent study Wu et al. (9) showed the relationship between

high job demand and WPV; in fact, an heavy workload and consequent high-job stress level among HCWs, showed being the cause of poor-quality medical care which was straightly linked to WPV perpetrated by dissatisfied patients towards HCWs.

Many studies emphasized also the relationship between occupational injuries among HCWs and workplace organizational factors related to work-shift schedules (i.e. work-shift duration, nightshift-work) (10, 11); among hospital registered nurses (RNs), Hopcia K. et al. (10) demonstrated that long working hours were related to injuries occurrence (not including WPV) and suggested the need of workplace policy targeted on work-shifts as a special concern in order to improve the safety and health of HCWs. Although these evidences, to date there is a lack of scientific data about the impact of work-shifts, including nightshifts, on the occurrence of WPV against ED HCWs.

The purpose of this study was to investigate the relationship between work shift schedules and WPV among RNs working on non-traditional shifts, including nights and 12-hour shifts.

Methods

The authors conducted a cross-sectional nested case-control analysis of data regarding the episodes of WPV perpetrated by patients or their relatives against RNs employed in three first-level EDs in Salento, south east of Italy, and staffing data for the same RNs in the period between January and December 2017; the aims of the study were to evaluate: 1) the relationship between consecutive workdays, nightshifts, cumulative hours and WPV towards RNs 2) the relationship between forward-rotating shift schedules (morning-afternoon-night) and WPV occurrence. For the aims of the present study, the authors analyzed the work shift prior to the date of each episode of WPV; in particular, consecutive workdays and cumulative hours worked in the previous 7 and 28 days, number of cumulative nights worked in the previous 7 and 28 days, direction of rotating shift schedules during the 28 days prior to the date of WPV, were detected. Nightshift was defined as a shift which included 1:00 am and 2:00 am as part of the shift. The analysis concerned 741 RNs (including 203 men); the mean age was 45,4 years (SD 2,9), mean length of service 23,8 years (SD 2, 5). The average volume of contacts for each ED was 42,000 patients per year. Cases were defined as the RNs who suffered a WPV from patients or their relatives during the 2017 year. If the assaulted RN reported more than one episode of WPV, all the WPV episodes were included in the study. Each case was matched with one RN employed in the same hospital, with similar demographic characteristic (unit type, job type, gender, age +/– 10 years). For this research the author examined the Occupational Prevention and Protection Service database based in the hospital and consisting of all incident reports and human resources informations. In this study, WPV perpetrated by patients or their relatives against HCWs was defined: "Any incident in which a person is abused, threatened or assaulted in circumstances relating to their work. This can include verbal abuse or threats as well as physical attacks" (12).

The statistical analysis included a logistic regression analysis to calculate the incidence and odds ratio (OR) with 95% confidence interval. All analyses were performed using SPSS software for Windows.

Forward-rotating shift was defined as a shift rotation in clockwise direction (begins during day, moves to evening and then to night); shift rotations were defined as backward-rotations if in counterclockwise direction: from day to night to evening shifts.

The study was performed as part of the obligatory evaluation of workplace violence, required by Italian Legislative Decree 81/08, and needed no formal approval by the local ethics committee.

Results

The study showed that in the analyzed period occurred 186 episodes of WPV; the one-year incidence of WPV was 29,30 per 100 Full Time Equivalent (FTE) positions. Twenty-one injured RNs reported more than one episode of WPV. No significant differences were found among cases and controls compared by cumulative hours and consecutive workdays worked in the 7 and 28 days prior to WPV occurrence. Cumulative nightshifts were significant for 3 or more nightshifts compared to working less than 3 nightshifts during the previous 7 days; additionally, RNs working 9 or more night-shifts showed higher risk of experiencing

WPV compared to RNs working less than 4 night-shifts in the previous 28 days (Table 1). WPV episodes occurred less frequently among HCWs adopting constant forward-rotating shift schedules compared to different shift schedules (i.e. back-rotating shift schedules or non constant forward-rotating shift schedules) during the last 28 days (OR=0,57; 95% CI= 0,35-0,91 p<0,05).

Discussion

The findings of the present study revealed the relationship between night-shift work and the occurrence of WPV against ED RNs; consequently, a strategic way in order to approach the issue of WPV should consider the impact of night shifts worked by RNs, with the aim to minimize the occurrence of WPV perpetrated by patients and their relatives towards RNs. In addition to the findings of many studies which demonstrated the link between shiftwork and consequent negative effects for both workers and healthcare enterprises (i.e. occupational stress, burnout, fatigue, sleeping difficulties, reduced work efficiency, poor performance, decreased job satisfaction, increased rates of absenteeism and turnover and increased accident and injury rates) (13-14), the present study showed the detrimental impact of frequent night shift work on ED HCWs security with regard to WPV occurrence, susceptible to be minimized through organizational interventions aimed at reducing the number of night shifts per RN up 2 nights a week and up 8 nights every 28 days. The analysis also showed that forward-rotating shift-work (shift rotation in the clockwise direction) was a protective shift schedule in regard to minimizing WPV occurrence when compared to shift schedules non systematically forward-rotating. This finding is consistent with literature (15), which revealed the systematic adoption of forward-rotating schedule as a strategic organizational way to better protect HCWs from occupational hazards rather than a backward-ro-

Table 1. Odds Ratios for number of night-shifts worked 7 and 28 days prior to the day of workplace violence (WPV) episodes

Number of night-shifts in previous 7 days (lenght ≥4 hours)	WPV cases N. 186 (%)	Controls N. 186 (%)	O.R. (95% CI)	P-value
0	7 (3,88)	12 (8,74)	1*	
1-2	88 (49,52)	123 (66,02)	1,23 (0,46-3,24)	>0,05
3-6	91 (46,60)	51 (25,24)	3,06 (1,13-8,26)	<0,05
Number of night-shifts in previous 28 days (lenght ≥4 hours)	NSSI cases N. 186 (%)	Controls N. 186 (%)	O.R. (95% CI)	P-value
< 4	49 (26,21)	64 (33,98)	1*	
4-8	49 (28,16)	73 (39,81)	0,88 (0,52-1,47)	>0,05
>8	88 (45,63)	49 (26,21)	2,35 (1,41-3,91)	<0,05

CI, confidence interval. *Referent category

tating schedule (shift rotation in the counterclockwise direction), or other rotating schedules.

Conclusion

In the present study the occurrence of WPV towards ED RNs appeared linked with shift-work schedules, including nightshifts. Healthcare managers should consider the need of organizational interventions aimed at adopting forward-rotating shift-work schedules and at avoiding nightshifts more than twice a week or eight monthly, with the aim to mimize the occurrence of WPV in EDs.

The present study suffers from some limitations: the shortness of the analyzed period and the smallness of the analyzed sample do not allow us to draw strong conclusions about the relationship between shift work schedules and occurrence of WPV. Although these limitations the analysis shows statistically significant increases in ORs for WPV among EDs HCWs working shift work schedules.

Highlights

1) In the present study the occurrence of WPV towards ED RNs appears linked with shift-work schedules, including nightshifts.
2) Organisational interventions are needed to better protect ED RNs from WPV
3) Healthcare managers should consider the need of organisational interventions aimed at adopting forward-rotating shift-work schedules and at avoiding nightshifts more than twice a week or eight monthly.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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