ISSN 2307-8960 (online)

World Journal of *Clinical Cases*

World J Clin Cases 2014 June 16; 2(6): 172-239

Published by Baishideng Publishing Group Inc

WJCC World Journal of Clinical Cases

A peer-reviewed, online, open-access journal of Clinical Cases

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World Journal of Clinical Cases

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WJCC World Journal of Clinical Cases

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Monthly Volume 2 Number 6 June 16, 2014

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World Journal of Clinical Cases (*World J Clin Cases*, *WJCC*, online ISSN 2307-8960, DOI: 10.12998) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

The primary task of *WJCC* is to rapidly publish high-quality Autobiography, Case Report, Clinical Case Conference (Clinicopathological Conference), Clinical Management, Diagnostic Advances, Editorial, Field of Vision, Frontier, Medical Ethics, Original Articles, Clinical Practice, Meta-Analysis, Minireviews, Review, Therapeutics Advances, and Topic Highlight, in the fields of allergy, anesthesiology, cardiac medicine, clinical genetics, clinical neurology, critical care, dentistry, dermatology, emergency medicine, endocrinology, family medicine, gastroenterology and hepatology, geriatrics and gerontology, hematology, immunology, infectious diseases, internal medicine, obstetrics and gynecology, oncology, ophthalmology, orthopedics, otolaryngology, pathology, pediatrics, peripheral vascular disease, psychiatry, radiology, rehabilitation, respiratory medicine, rheumatology, surgery, toxicology, transplantation, and urology and nephrology.

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ISSN
ISSN 2307-8960 (online)

LAUNCH DATE
April 16, 2013

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EDITORIAL OFFICE
Jin-Lei Wang, Director
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World Journal of Clinical Cases
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
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PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
June 16, 2014

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DOI: 10.12998/wjcc.v2.i6.172

World J Clin Cases 2014 June 16; 2(6): 172-193
ISSN 2307-8960 (online)
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REVIEW

Role of human papillomavirus in oropharyngeal squamous cell carcinoma: A review

Robbie SR Woods, Esther M O'Regan, Susan Kennedy, Cara Martin, John J O'Leary, Conrad Timon

Robbie SR Woods, Conrad Timon, Department of Otolaryngology, Royal Victoria Eye and Ear Hospital, Dublin 2, Ireland
Esther M O'Regan, John J O'Leary, Department of Histopathology, St. James's Hospital, Dublin 8, Ireland
Susan Kennedy, Department of Histopathology, Royal Victoria Eye and Ear Hospital, Dublin 2, Ireland
Cara Martin, John J O'Leary, Molecular Pathology Research, Coombe Women and Infants University Hospital, Dublin 8, Ireland
Author contributions: Timon C initiated the project and contributed to the clinical aspects of the paper; O'Regan EM, Kennedy S, Martin C and O'Leary JJ designed the research and contributed to the separate headings in the paper; Woods RSR wrote the paper; Timon C, O'Regan EM, Kennedy S, Martin C and O'Leary JJ revised the article and approved the final version.
Correspondence to: Robbie SR Woods, BA, MB, BCh, BAO, MRCSI, MCh, Department of Otolaryngology, Royal Victoria Eye and Ear Hospital, Adelaide Road, Dublin 2, Ireland. robbiewoods@rcsi.ie
Telephone: +353-1-6644600 Fax: +353-1-6761858
Received: March 2, 2014 Revised: April 21, 2014
Accepted: May 16, 2014
Published online: June 16, 2014

Abstract

Human papillomavirus (HPV) has been implicated in the pathogenesis of a subset of oropharyngeal squamous cell carcinoma. As a result, traditional paradigms in relation to the management of head and neck squamous cell carcinoma have been changing. Research into HPV-related oropharyngeal squamous cell carcinoma is rapidly expanding, however many molecular pathological and clinical aspects of the role of HPV remain uncertain and are the subject of ongoing investigation. A detailed search of the literature pertaining to HPV-related oropharyngeal squamous cell carcinoma was performed and information on the topic was gathered. In this article, we present an extensive review of the current literature on the role of HPV in oropharyngeal squamous cell carcinoma, particularly in relation to epidemiology, risk factors, carcinogenesis, biomarkers and clinical implications. HPV has been established as a causative agent in oropharyngeal squamous cell carcinoma and biologically active HPV can act as a prognosticator with better overall survival than HPV-negative tumours. A distinct group of younger patients with limited tobacco and alcohol exposure have emerged as characteristic of this HPV-related subset of squamous cell carcinoma of the head and neck. However, the exact molecular mechanisms of carcinogenesis are not completely understood and further studies are needed to assist development of optimal prevention and treatment modalities.

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Key words: Human papillomavirus; Human papillomavirus; Oropharynx; Oropharyngeal; Squamous cell carcinoma; Head and neck; Oncology

Core tip: Human papillomavirus has been accepted as a causative agent in a subset of head and neck squamous cell carcinoma (SCC), particularly of the tonsils and base of tongue. Importantly, there is an increasing incidence of this subset of patients, who demonstrate improved prognosis and may respond more favourably to treatment. Similarities and differences are evident between cervical and oropharyngeal human papillomavirus-related SCCs and the comparison between these tumours warrants further investigation to better understand the disease process.

Woods RSR, O'Regan EM, Kennedy S, Martin C, O'Leary JJ, Timon C. Role of human papillomavirus in oropharyngeal squamous cell carcinoma: A review. *World J Clin Cases* 2014; 2(6): 172-193 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i6/172.htm DOI: http://dx.doi.org/10.12998/wjcc.v2.i6.172

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is

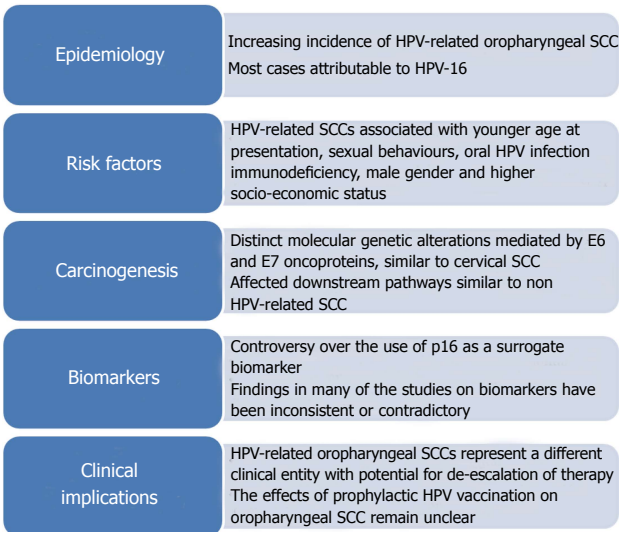

Figure 1 Summary of key points. HPV: Human papillomavirus; SCC: Squamous cell carcinoma.

the sixth most common type of cancer worldwide with approximately 633000 new cases diagnosed and 355000 deaths annually[1]. Over the past 10-15 years, the traditional paradigms of HNSCC have been changing significantly. It has emerged as a heterogeneous group of diseases, with distinct molecular genetic changes[2,3].

Human papillomavirus (HPV) has been linked to the pathogenesis of squamous cell carcinoma (SCC) since the 1970s[4] and, in 1995, it was recognised by the International Agency for Research on Cancer (IARC) that high risk HPV types 16 and 18 were carcinogenic in humans[5]. The role of HPV in cervical cancer is well described[6], however high risk HPV types are also linked with other ano-genital tumours and with SCCs of the head and neck[7,8], as well as potentially playing a role in cutaneous SCCs[9]. HPV accounts for roughly 4.8%-5.2% of the total global cancer burden, making it the highest among all viruses[10,11].

Since it was first suggested in 1983[12] and first identified in 1985[13], HPV infection has been increasingly recognized as a major aetiologic factor for HNSCCs, particularly a subset that arise from the oropharynx, mostly the base of tongue and palatine tonsils[14-16]. This subset is seen as a distinct clinicopathological entity in comparison to the traditional smoking and alcohol related HNSCCs[16-20]. Specific genetic changes induced through HPV E6 and E7 protein expression define this subset[21-23]. In contrast, tobacco associated HNSCCs are usually more genetically diverse[24]. HPV-related tumours of the oropharynx display specificity of HPV to the tumour cell nuclei[16], integration of HPV DNA into the host cell[16,25] and high viral copy numbers[26], giving evidence for the functional role of HPV in the pathogenesis of these tumours.

HPV-related SCC tends to display unique histology characterized by poorly differentiated, non-keratinising morphology with a basaloid appearance[17,27]. Nevertheless, even some true basaloid squamous cell carcinomas of the oropharynx have demonstrated HPV-positivity[28], and other variants such as papillary SCC, adenosquamous carcinoma, lymphoepithelial carcinoma-like tumours and small cell carcinoma have been associated with HPV infection[29-34].

It is estimated that the probability of a cancer of the oropharynx being attributable to HPV is five times higher than the oral cavity, larynx or hypopharynx[35], with HPV-related oropharyngeal SCC being described as an epidemic[36-40]. Current data from studies that assessed in situ hybridization or HPV E6/E7 mRNA suggest that HPV-related HNSCC is rare in the oral cavity, larynx, hypopharynx and other HNSCC sites[35], however the role of HPV in non-oropharyngeal sites remains unclear[41] and a causative relationship at these sites has not been established[42].

We review the current literature regarding HPV-related oropharyngeal tumours with regard to epidemiology, risk factors, carcinogenesis, biomarkers and clinical implications. A summary is shown in Figure 1.

HUMAN PAPILLOMAVIRIDIAE

HPV is an epitheliotropic, non-enveloped DNA virus measuring approximately 55 nm in diameter, and carries a single molecule of circular double-stranded DNA, consisting of approximately 8000 base pairs[43]. The genome is broken down into three regions which consist of a long control region (LCR), an early (E) region and a late (L) region. There are eight genes in the E region and two in the L region. These genes in E and L encode viral proteins while LCR is an upstream non-coding regulatory region containing the origin of viral DNA replication and transcriptional regulatory elements.

At present, over 200 different genotypes of papillomaviridiae, characterized by at least 10% nucleotide divergence in capsid gene (*L1*)[44], have been identified by various techniques[45]. These can be classified according to similarities in their DNA sequences. They have also been grouped into mucosal (mostly of the alpha genus) or cutaneous (mostly of the beta genus) types based on their tropism for specific epithelia and they can be classified into low and high risk types based on their capacity to promote malignant transformation in host cells. Of these, HPV 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 and 82 are examples of those classified as high risk viruses, detectable in high grade squamous intraepithelial lesions in the cervix or in invasive cancer; while HPV 6, 11, 40, 42, 43, 44, 54, 61, 72, 81, and 89 can be considered as viruses with low oncogenic risk and can be isolated from low grade epithelial lesions of the cervix. There remain a number of HPV types that are potentially high risk with an unknown oncogenic potential. There exists some degree of intratypic variation[46,47], which may also relate to pathogenesis[48-50], as well as geographic variation in genotype prevalence[46,51].

HPV is one of the most powerful human carcinogens. The E6 and E7 genes produce E6 and E7 oncoproteins,

which confer the virus with oncogenic potential through their inhibitory effects on p53 and retinoblastoma (Rb) proteins, more of which is discussed later.

EPIDEMIOLOGY

HNSCC includes tumours from a number of subsites, of which the oropharynx accounts for approximately 10%[52]. Worldwide, there were an estimated 85000 new cases of oropharyngeal SCC in 2008, of which 25.6% (22000) were estimated to be HPV-related[53]. Of the HPV-related cases, more than three quarters (17000) were estimated to be male.

Genotypes of oncogenic HPV found in cervical cancer in order of prevalence are 16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51 and 56[54]. However, the distribution of HPV types differs somewhat in oropharyngeal when compared to cervical cancers[55]. A systematic review found that HPV-16 was present in 95.7% of HPV-related oropharyngeal SCC, but only 73.9% of HPV-positive non-oropharyngeal HNSCCs[56], while only approximately 61% of cervical cancer display HPV-16[57]. While a significant number of other oncogenic HPV types are found in cervical cancer, only a small proportion of oropharyngeal cancers may be caused by additional HPV types such as 18, 31, 33, 35, 52 and 58[58,59]. HPV-16 is the commonest genotype found in oral cavity infection[60], while it constitutes over 90% of the genotype distribution in tonsil cancers[61].

Prevalence of oral high risk HPV infection in the general population is reported at 3.5%-3.7%[62,63], with higher rates for those also infected with HIV[64]. A systematic review of the literature in 2005 reported detection of HPV DNA in 35.6% of oropharyngeal tumours[65]. However, there exists a wide geographic variation, with a reported prevalence as high as 72%[59] in North America compared to 17% in southern Europe[53], 12.6% in Taiwan[66] and even as low as 4.4% reported in central Europe and Latin America[67]. Some of these figures are based on the assumption that detection of high-risk HPV DNA in tumour tissue signifies cancer attributable to HPV, however this does not delineate from the effects of tobacco exposure and alcohol in these cases. It has been recorded that HPV accounts for approximately 7.7% and 2.2% of all cancer cases in developing and developed countries, respectively[10]. The variations could partly be explained by geographic and temporal heterogeneity in sexual behaviours and tobacco exposure[41]. A more recent systematic review in 2012 reported a prevalence of HPV in oropharyngeal SCC of 59.9% in the United States, compared to 39.7% in Europe and 32.5% in the rest of the world[56]. There are limited data from less developed regions, but the incidence appears much lower.

Despite the variation in prevalence, case control studies conducted around the world show strong and consistent associations of markers of HPV exposure with risk of oropharyngeal cancers, even after adjustment for important HNSCC risk factors such as age, gender and tobacco and alcohol use[41].

While incidence of other HNSCCs has decreased over the past two decades, correlating with decreased tobacco use, the age-adjusted incidence of oropharyngeal SCC has been increasing in this same period[68,69], particularly of the base of tongue and tonsil region[70]. Meanwhile the population-level incidence of HPV positive oropharyngeal SCC increased by 225% between 1988 and 2004, with a concomitant decline by 50% for HPV-negative oropharyngeal SCC[59]. A particularly steep rise of over 70% has been reported for prevalence of HPV-related oropharyngeal SCC in the past decade, with prevalence in Europe increasing at a faster rate than North America[56]. This rise further emphasises the predilection of HPV for the oropharynx and suggests that it plays a less significant role in other HNSCCs.

With the rise in HPV-related oropharyngeal SCC coupled with the decline of HPV-related cervical SCC, it has been suggested that the annual numbers of HPV-related oropharyngeal cases could soon surpass that of cervical cancer[41].

RISK FACTORS

HNSCCs, including those of the oropharynx, have traditionally been strongly associated with patients who have a long history of heavy smoking and alcohol consumption, with previous studies clearly showing a dose-response relationship with the frequency and duration of tobacco and alcohol exposure[71]. Age of onset is generally in an older age group (usually seventh decade) in these traditional HPV-negative oropharyngeal SCCs. Other risk factor associations with these tumours include poor oral hygiene[72,73], a diet low in fruit and vegetable consumption[74,75] and chronic inflammatory disease in the oral cavity[76-78].

Age

The distinct subset of HPV-positive oropharyngeal SCCs generally present at a younger age, averaging a few years lower than HPV-negative tumours[39]. Although phenotypically similar to those in older patients, HNSSCs developing in younger patients are undoubtedly different at a genetic level with both germline and somatic differences seen[3,79-82]. One study showed that patients under 55 had a 3.4-fold higher risk of infection with carcinogenic HPV[83], while a strong association has been demonstrated with HPV-16 infection and tonsillar cancer in males under 40 years old[84]. Increasing incidence of oropharyngeal SCC is seen in those aged under 60[85], with a particularly steep rise seen between the ages of 50-59[86], although it is possible this may be due to other risk factor exposures in this birth cohort.

Sexual behaviours

HPV-related oropharyngeal SCCs also show strong associations with sexual behaviours, correlating with disease[87]. In a large number of studies, both HPV-positive

HNSCCs and oropharyngeal SCCs have been strongly associated in comparison to other HNSCCs with number of lifetime sexual partners, number of vaginal, oral and anal sexual partners, young age at first intercourse/earlier sexual contact and history of sexually transmitted diseases, including genital warts[27,83,87-93]. After adjusting for HPV-16 serology, the associations in a case-control series were no longer significant, suggesting that sexual behaviours can be seen as a surrogate for HPV-16 exposure[27].

Data from a number of developed countries show that markers of high-risk sexual behaviours, such as earlier ages of sexual debut, practice of premarital sex, average number of lifetime partners, and practice of oral sex, have all increased among recent birth cohorts[94].

Oral HPV infection

Oral HPV is predominantly acquired *via* sexual transmission and oral HPV prevalence has been associated with some of the above sexual behaviours. Studies have demonstrated increased HPV acquisition around sexual debut with oral HPV prevalence of 1.5% in 12-15 year olds, 3.3% in 16-20 year olds and 4.5%-6.9% in healthy adults[62,63,89,95]. Higher oral HPV prevalence has been reported in women with cervical HPV infection[96,97], and people infected with Human Immunodeficiency Virus (HIV)[96,98]. Several studies and some case reports have described concordant oral HPV infection between couples[99-102], however preliminary results from the HPV oral transmission study in partners over time (HOTSPOT) have not backed up these findings.

It has even been suggested that non-sexual HPV transmission through kissing may be possible[95,103], as well as intrapartum transmission[104] and transmission during laser surgery[105]. In itself, oral HPV-16 infection is a strong risk factor for oropharyngeal cancer, while the relationship is not necessarily clear for oral SCCs[106,107]. However, oral HPV prevalence is lower than cervical, perhaps explained by a lower proportion in oral-genital than genital-genital partners[55], but the natural history of HPV infection in the oral cavity appears similar to cervical infections[108]. Although type-specific concordance is low, HPV infection of the cervix and oral cavity are not independent[109] and so cervical HPV infection could be considered a risk factor for oral cavity HPV infection. Although the full natural history of HPV infection in the oral cavity and oropharynx is not entirely understood, there is an estimated incidence of 4.4% per year with most infections being cleared within one year[110]. However, changing sexual practices are potentially leading to higher rates of infection that could become recalcitrant to immune responses.

Tobacco and alcohol exposure

Evidence of a role for tobacco exposure and alcohol use in HPV-related oropharyngeal SCCs and in oral HPV infection is equivocal, with some studies reporting positive association and suggesting smoking-induced immunosuppression or potentiation of carcinogenesis could play a role, while others report no association[41]. A role for tobacco smoking in cervical cancer, however, has been demonstrated, although this association becomes weak after adjustment for sexual and reproductive factors[111]. In comparison to traditional HNSCCs, these patients are less likely to have excessive tobacco exposure and alcohol use[16,88,112], however HPV-related oropharyngeal SCCs do occur in both in those with tobacco exposure and alcohol use and in those without. It is highly plausible that tobacco exposure potentiates the effects of HPV carcinogenesis[113] but a role in the causation of HPV-related oropharyngeal SCCs has not been definitively determined from available evidence[35]. Marijuana use has also been associated with oropharyngeal SCCs[87,114], however after adjustment for sexual behaviour variables in one study, this disappeared[62].

Gender

Both HPV-related and non HPV-related HNSCC exhibit male predominance at a ratio of approximately 3:1. In tobacco and alcohol related HNSCC, this difference has decreased particularly as trends in smoking have changed, with 43% of men and 30% of women smoking in 1974 compared to 26% of men and 21% of women in 2000[115]. Nonetheless, the difference still remains for HPV-related HNSCC and the reason for this is uncertain. The male predominance exhibited cannot be fully explained by difference in sexual behaviours, which suggests potential biologic differences between men and women[41,116], or that some male characteristic preferentially predisposes to cancer of the oropharynx[117]. It has been suggested that hormonal differences[55,118] or the potential protective immunity from seroconversion in response to cervical HPV infections among women[119,120] may play a role. Although not all studies agree[63], the majority of studies report that oral HPV infection is more common in men than women[62,121,122]. It has also been suggested that transmissibility of oral HPV may be higher for men performing oral sex on women, possibly due to a higher HPV copy number in the vagina/cervix[94].

Immunodeficiency

Immunodeficiency is a risk factor for a large number of tumours and HPV-related oropharyngeal SCC is included in that. For example, it is reported that patients infected with human immunodeficiency virus (HIV) have a 2-6 times increased risk of HPV-related HNSCC[123,124], although they are at greater risk of ano-genital SCCs than oropharyngeal[125]. It has been demonstrated in cervical cancer patients that immunosuppression leads to HPV persistence and disease progression[126-128]. The association of a deficient immune system with increased HPV-related HNSCC may partly explain any potential association with tobacco exposure due to the immunosuppressive effects of smoking[129], with one paper demonstrating a reduced antibody response in smokers[130].

Socio-economic status

HNSCCs have been associated with patients from a low socio-economic group for many years[131]. However, HPV-

related oropharyngeal SCCs are associated with patients who are from a higher socio-economic group and who have a better performance status[132,133], although this has been refuted in one study[116]. Nonetheless, white males seem to be particularly at risk, with a rise in incidence reported in this group alone[59,85,116]. HPV positivity in oropharyngeal cancer is lower in African Americans than in other racial groups, with poorer survival in this racial group from oropharyngeal SCC, because a higher proportion is related to tobacco and alcohol exposure[134,135].

HPV serology

There is a strong association between serologic evidence of HPV infection and HNSCC risk, even after adjustment for other HNSCC risk factors[106]. One study has even shown a temporal association, with pre-diagnostic serum samples from ten years prior that were positive for HPV-16 capsid antibodies conferring an increased risk of oropharyngeal SCC of 14.4[136], while patients with pre-diagnostic E6 seropositivity had a significantly higher risk of oropharyngeal cancer in another study[137].

It is evident that a number of factors can facilitate or increase the risk of HPV-related oropharyngeal SCCs. This includes oral HPV infection, male gender, younger age, white race, immunosuppression and a variety of sexual behaviours. Differences in sexual behaviours across age and gender and consequent HPV exposure risk could account for the rapidly increasing incidence of HPV-related oropharyngeal SCCs among younger patients. Interestingly a separate specific subgroup of younger females with non HPV-related oral cavity SCCs has also been identified[138].

CARCINOGENESIS

The model for development of SCC involves exposure to carcinogens over time leading to progressive genetic and epigenetic changes that accumulate and lead to premalignant and eventually malignant lesions. However, HNSCC is a heterogeneous disease with a number of subtypes described, based on histological appearance, and supported by different gene expression profiles[139,140]. Squamous cell carcinomas from different sites in the body share a number of molecular characteristics but recent whole-exome sequencing[141-144] has helped to characterise the specific molecular pathogenesis of HNSCC with roles identified for tumour suppressor pathways including p53, Rb/INK4/ARF and NOTCH[145]. A role for cancer stem cells in HNSCC is likely, based on recent evidence[146-149], and further study of these progenitor cells will help to elucidate mechanisms of carcinogenesis.

Recent deep-sequencing studies on the HNSCC oncogenome have demonstrated a vast number of diverse genetic alterations, however most of these converge on four targetable molecular pathways[150]; mitogenic signalling and in particular amplification or up-regulation of epidermal growth factor receptor (EGFR) and the downstream pathway of phosphoinositide 3-kinase (PI3K)/mTOR as well as PTEN inactivation, each leading to pathways involving proliferation, DNA repair, survival and spread; defective differentiation involving NOTCH signalling alterations; cell cycle de-regulation involving inactivation of CDKN2A (encoding p16 INK4A) tumour suppressor gene and CCND1 (encoding CYCLIN D1) amplification; genomic instability involving loss of TP53, which occurs in a large percentage of non HPV-related HNSCC and is the single most common mutational event, and other genes related to DNA damage recognition and repair. It is possible that smoking and alcohol affect distinct genes[151], giving further evidence for a synergistic effect of tobacco and alcohol exposure in relation to HNSCC carcinogenesis.

HNSCC usually displays field cancerisation, a term first coined in 1953[152], whereby specific genetic alterations can be widely distributed throughout the mucosa lining the aerodigestive tract even in the absence of overt histopathologic changes of malignancy[25]. Only a minority of precancerous fields in the oral cavity are recognised as leukoplakia or erythroplakia[153] and only 6%-36% of patients with leukoplakia or erythroplakia go on to develop oral SCC[154], particularly those demonstrating aneuploidy[155,156]. The accumulation of further genetic changes in precancerous fields leads to the development of SCC, with presence of field change leading to a higher risk of multiple synchronous or metachronous primary tumours. Exposure to carcinogens bring about these field changes, however evidence for a field effect is lacking for HPV-related SCC[157] and the risk of second primary malignancy in oropharyngeal SCC has markedly decreased over time[158], with the mutation rate of HPV-positive tumours only approximately half of that found in HPV-negative HNSCC[141,142].

Specific differences in chromosomal alteration and gene transcription have been identified between HPV and non HPV-related HNSCCs[21,22,159,160]. TP53 mutations, loss of 9p21, hypermethylation of 14-3-3σ and RASSF1A promoters and overexpression of cyclin D are all common in non HPV-related oropharyngeal SCCs, while pRb levels are normal and p16 is often decreased[161,162].

In the cervix, after initial infection at the transformation zone, viral genomes are maintained as episomes in the basal layer, with viral gene expression being tightly controlled as the infected cells move toward the epithelial surface[163]. Subsequent high-grade neoplasia represents an abortive infection in which viral gene expression becomes deregulated and the normal life cycle of the virus cannot be completed. The squamous epithelium in the cervix and the head and neck derive embryologically from endoderm and are susceptible to metaplasia[164]. In the head and neck, there is a predilection for HPV-positive tumours to occur in the reticular crypt epithelium of palatine and lingual tonsils and head and neck sites with mucosa associated lymphoid tissue[25,165,166]. It is possible that this occurs due to the particular microanatomy of the crypts, where there are breaks in the non-keratinising squamous epithelium that could allow viral entry, while a microabrasion theory of entry to basal cells at other head and neck sites has been proposed. Entry may be facilitat-

ed by M-cells lining the crypt epithelium[167], as with other viruses[168,169]. Another theory postulated is an influence on HPV carcinogenesis from increased cytokines related to nearby lymphoid tissue[170]. The recent observation of a distinct set of embryonic cells at the squamocolumnar junction of the cervix, which seem to confer a particularly high risk of malignancy, has led to a "top-down" theory of malignancy at this site, although it remains to be seen if this model translates to the oropharynx[171-173]. Despite being full of lymphatic tissue, the tonsils are known to harbour pathogenic viruses such as Epstein Barr virus, adenoviruses and herpes simplex virus[174], and it is the mechanisms of immune evasion that allow persistent infection and carcinogenic potential at these sites, hence immunosuppressed individuals are particularly at risk.

From cervical models, we understand that most HPV infections last no more than a few months and are eliminated by the immune response, with 90% of infections cleared within two years, although high risk HPV tends to persist longer than low risk[175,176]. Once immune evasion is established, integration of HPV DNA into the cellular genome likely represents a critical step for malignant transformation in those individuals who harbour HPV in their tonsils[25], with HPV integration representing a stochastic process resulting in clonal selection of aggressively expanding cells that display altered gene expression of integrated HPV genomes and potential perturbations of cellular genes at or near viral integration sites[177]. Viral integration can also lead to loss of E2-mediated inhibition of viral oncoprotein expression[178]. Furthermore, it has been shown that this HPV DNA integration is consistently centred on tonsil crypt epithelium[25], however the factors allowing transformation from episomal HPV infection, whether active or latent, to DNA integration remain poorly understood. It has also been noted that much of the HPV that is detected in oropharyngeal cancers seems to be episomal.

Based on cervical cancer models, high-risk HPV can induce genetic changes in a small number of those with persistent infection which leads to precancerous lesions, a fraction of whom will develop cancer many years after the original infection. While HPV-related precursor lesions in the oral cavity have been identified[179], there is an absence of detectable precancerous lesions in the oropharynx[41], perhaps related to the difficulty in assessing and sampling deep tonsillar crypts, the predominant location of HPV-related SCCs[180,181]. Nevertheless, HPV-related oropharyngeal SCCs present with distinct molecular profiles, more comparable to cervical SCC than to non HPV-related HNSCC[55]. Infection with HPV is likely an early oncogenic event in HNSCCs. The viral oncoproteins E6 (151 amino acids) and E7 (98 amino acids) of high risk HPV types, particularly HPV-16, are implicated as the drivers of transformation in HPV-related oropharyngeal SCCs[182]. These proteins help to re-program postmitotic terminally differentiated epithelial cells to re-enter the cell cycle and express proteins that are required for viral genome replication[183]. They also disrupt a number of cellular mechanisms through a wide variety of downstream effects.

The E5 oncoprotein co-operates with E6 and E7 to promote proliferation of infected cells and is likely to facilitate malignant progression[184], although this process is likely to take place in the early stages of carcinogenesis because viral integration frequently leads to loss of E5 gene expression[185]. Transcription of E6 and E7 viral oncogenes can occur when the virus is episomal however, in cervical SCC, alteration of E2 on integration may facilitate increased expression of E6 and E7 oncogenes, although this may not be the case in oropharyngeal SCC[186]. Viral integration is thought to play an important role in cervical SCC but the relevance of viral integration is not fully clear in oropharyngeal SCC[187]. Some studies suggest that viral integration in the tonsillar crypts plays an important role in carcinogenesis[165,188], which may explain the predilection of HPV-related HNSCCs at this site, while other studies suggest that episomal HPV alone contributes to the development of most oropharyngeal SCCs in contrast to SCCs of the cervix[186,187].

In cervical lesions, it is not possible to predict tumour progression based on HPV viral load[189]. It has been suggested that high HPV viral load (at least one HPV copy per tumour cell) in oropharyngeal SCC predicts active HPV infection[190-192]. The proportion of HPV-positive SCCs with high viral load varies between studies from 33%-77.5%[59,190]. It is possible that in cases of low viral load that HPV presence is coincidental and alternative mechanisms of carcinogenesis are implicated. However, gene expression varies widely and so a constitutive rather than a high expression of viral oncogenes may be all that is required for HPV-related oropharyngeal carcinogenesis[187].

The major role of E6 oncoprotein is induction of ubiquitin-mediated proteolysis, through E6 associated protein, leading to degradation of tumour suppressor p53. As p53 usually facilitates repair to damaged host DNA by arresting cells in the G1 phase (or else inducing apoptosis), E6 expressing cells face increased mitotic stress and genomic instability[193]. E6 aids cellular proliferation by up-regulating transcription of telomerase[194] and also, through the presence of the PDZ binding motif, high risk HPV E6 proteins bind to a number of PDZ domain containing proteins with presumed tumour suppressor activity that have diverse functions[183,195]. E6 also targets the Wnt and Notch signalling pathways[183].

The E7 oncoprotein causes cell cycle disruption by binding and inactivating tumour suppressor proteins of the retinoblastoma family (pRb) that regulate cellular senescence. E7 thereby causes cell proliferation through abnormal entry into the S-phase by the overexpression of released transcription factor E2F[196]. This functional inactivation of pRB also results in overexpression of p16 tumour suppressor protein, which is a CDK4A inhibitor, allowing the use of p16 as a surrogate marker for HPV-related oncogenesis[39,197-200], which will be discussed further below. E7 proteins also alter cell cycle control through interactions with histone deacetylases, cyclins

and cyclin-dependent kinase inhibitors[201].

Animal models suggest that E7 is the dominant HPV oncoprotein in HNSCC[202], but both E6 and E7 directly impact upon a number of apoptotic mechanisms; interaction with extracellular matrix adherence proteins to allow anchorage independent growth; interaction with cell surface receptors to resist cytokine induced extrinsic apoptosis; and interaction with proteins involved in interferon signalling and interleukin to allow immune evasion[201,203].

Genomic instability underpins the development of dysplasia, malignancy, invasion, and metastasis in cancers[204]. While aberrant proliferation induced by E7 is facilitated by suppression of apoptosis by E6 mechanisms, it is the additional functions of E6 and E7 to induce genomic instability by multiple mechanisms that lead to chromosomal mutations. These include centrosome abnormalities or spindle checkpoint failure leading to polyploidy, aneuploidy and chromosomal rearrangement[205,206], direct DNA damage[207] (which also occurs with viral integration[208]), variation in the Fanconi anaemia DNA repair pathway and induction of the ATM-ATR DNA damage repair pathway with concomitant disruption of checkpoint control mechanisms[201]. Tobacco exposure also causes genomic instability and so may help to induce malignancy on the background of E6 and E7 effects, allowing for a role of tobacco exposure in the potentiation of HPV-related HNSCC, which has been suggested from mouse models[209].

Different patterns of DNA methylation have been demonstrated between HPV and non HPV-related HNSCCs, with methylation patterns in HPV-related HNSCCs more analogous to cervical SCC patterns than non HPV-related HNSCCs[210]. Excess DNA methylation could be recruited by the integrated viral genome rendering it invisible to host immune responses or it could be an attempted defence mechanism by the host cell[210]. HPV-related HNSCCs also have a distinct miRNA profile, also more analogous to cervical SCCs, in comparison with non HPV-related HNSCCs[211]. Furthermore, differences in DNA methylation rate have been identified between HNSCCs in tobacco users versus nonusers as well as specific mRNA and microRNA clusters[212].

While distinct methods of carcinogenesis are evident between HPV-related and non HPV-related HNSCCs, the effects on downstream pathways are often the same, such as in the case of mTOR inhibition, either from TP53 mutations in tobacco related cases or from E6 induced degradation of p53 in HPV-related cases[150]. It is also important to note that E6 and E7 proteins expressed in low risk HPV types do not induce the same changes and that HPV present in some HNSCCs may exist as a latent passenger virus with no transcriptional activity[213,214]. New roles for HPV oncoproteins are continually being identified, offering many future potential therapeutic targets[215]. In any case, there is a distinct group of HPV-related tumours arising from the epithelium of lymphoid tissue characterised by viral oncoprotein expression, rather than SCCs that arise on a background of a long history of somatic mutations due to carcinogenic exposures. A proposed model of carcinogenesis in HPV-related oropharyngeal SCC is shown in Figure 2.

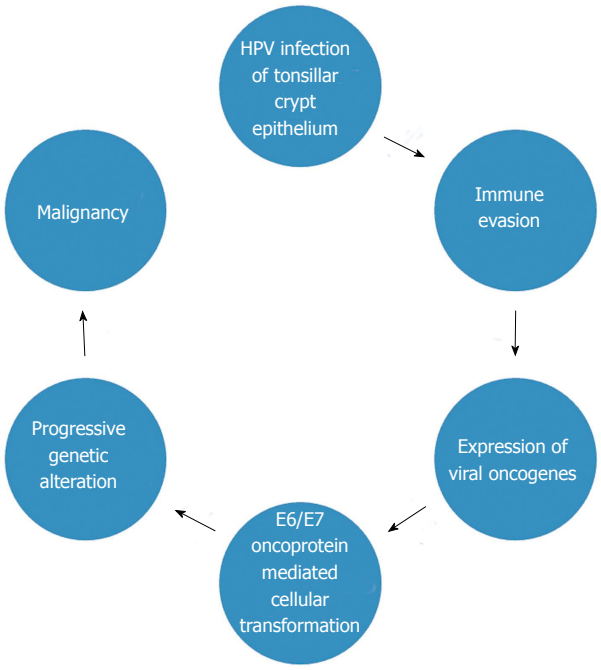

Figure 2 Proposed Theory of human papillomavirus-related carcinogenesis. HPV: Human papillomavirus.

BIOMARKERS

Studies have had difficulty identifying clinically useful biomarkers in HNSCC[216]. A high degree of heterogeneity is evident in HNSCC, with different prognosis described for different subsets of tumours. This includes a favourable prognosis for the growing cohort of HPV-related SCCs, particularly oropharyngeal[217], often despite a more advanced presentation. This is due to a number of factors including the sensitivity of this subset to chemoradiation[133], the lower likelihood of loco-regional recurrence[217] and a younger cohort of patients with fewer comorbidities as well as a possible decreased risk of second primary tumours.

HPV status and p16 status have each proven useful as biomarkers in HNSCC. The tumour suppressor p16 binds to the cyclin D1 CDK4/CDK6 complex, thereby helping to keep the Rb protein in its active hypophosphorylated form. With pRb functionally inactivated by the binding of HPV E7 oncoprotein, p16 expression is upregulated by its corresponding gene being released from transcriptional inhibition. In non HPV-related HNSCC, downregulation or loss of p16 protein expression is a common early event and is associated with a worse prognosis, consistent with the tumour-suppressor role it has[204], and oral cavity and hypopharyngeal SCC show lower levels of p16 positivity[218,219]. However, a strong correlation has been observed in numerous studies between integrated HPV detection and p16 protein overexpression. As such, p16 has been adopted as a surrogate biomarker for HPV-related HNSCC[39,197-200,220,221], with

immunohistochemistry for $p16^{INK4A}$ now routinely performed in many laboratories and guides for interpretation have been described[199].

Not only can p16 act as a surrogate biomarker for HPV status, with 46%-98% of HPV positive oropharyngeal SCCs demonstrating p16 positivity on pooled analysis[200], but, with 3%-51% of HNSCCs being p16 positive and HPV negative[200], p16 status can also act as an independent prognosticator, regardless of HPV status[222-225], although not all studies agree on the specific effect[214]. Overexpression of p16 has been found in normal tonsillar tissue[25,226] and HPV negative tumours, with dysregulation of epigenetic control or multiple transcription factors being other mechanisms that lead to aberrant expression of p16[227], some of which are associated with non HPV-related HNSCC carcinogenesis. The lack of clarity on p16 expression and discrepancies in interpretation of p16 IHC have led to controversy surrounding its use as a surrogate biomarker.

Overexpression of p16 is not evident in a subgroup of HNSCC with active HPV infection[228], 2%-54% in pooled analysis[200]. With overexpression of $p16^{INK4A}$ thought to represent activity of viral oncogenes, it is possible that HPV positive/p16 negative may represent latent HPV infection, which could explain why HPV positive/p16 negative HNSCCs have a slightly worse prognosis[229,230]. Therefore, by combining testing for HPV DNA positivity and p16 overexpression, one can eliminate cases related to inactive infection, improving specificity of p16 a surrogate biomarker for detection of biologically relevant HPV infection[200]. This has been shown to be as reliable as detection of HPV E6/E7 mRNA expression by polymerase chain reaction, which is considered the gold standard of testing for transcriptionally active virus because HPV-negative HNSCCs and HPV-positive/E6 and E7 mRNA-negative HNSCCs show similar survival curves[191]; however E6/E7 mRNA is only used in a small number of centres and is mostly restricted to the research laboratory[231]. Equivalent detection may also be possible with HPV mRNA ISH and this may be of more practical clinical use[232,233]. However, there is currently a great degree of heterogeneity of HPV assessment techniques used in clinical practice depending on location[234].

A large number of alternative prognostic or predictive biomarkers in HNSCC have been studied, such as EGFR, cyclin D1, Bcl-2, cyclin-dependent kinase inhibitor p27, MCM7, DSG3, vascular endothelial growth factor, p53, ERCC1, RRM1, β-catenin and MET[209,235-240]. Some examples of studies on biomarkers related to treatment response found MMP-7 and EGF to be predictive markers of, respectively, resistance to cisplatin and poor response to cetuximab[241-243], while survivin overexpression predicted improved response to radiotherapy[244]. However, findings in many of the studies on biomarkers have been either inconsistent or often even contradictory. Some biomarkers have not been studied in sufficient detail to draw a firm association[216]. However, EGFR positivity has been associated with poor survival in HNSCC in a number of studies[245-247], including in HPV-related HNSCCs[167,235] (although these tumours generally tend not to overexpress EGFR[248]), and an EGFR-targeting antibody, cetuximab, has shown benefit in combination with radiotherapy for patients with HNSCC[249] so is approved for clinical use alone or in combination with radiotherapy or chemotherapy. Evidence suggests p16 may be useful in the context of analysing treatment response to cetuximab[250]. Besides EGFR inhibition, new molecular targeted therapies that have an effect on other activated molecular signalling pathways such as mTOR, Src kinase and IGF-1R inhibitors are being developed[251].

In HPV-related oropharyngeal SCC, there is the potential for translation of cervical biomarkers given the similarities in carcinogenesis between these sites. New biomarkers are continually emerging from molecular biological research which are of as yet uncertain relevance, such as recently discovered distinct squamocolumnar junction-related biomarkers[171-173].

It has been suggested that the programmed death 1 (PD-1):PD-L1 pathway plays a role in HNSCC, particularly in HPV-related oropharyngeal cases[252], by facilitating HPV-related carcinogenesis in an immune-privileged site[253]. Furthermore, an investigation into a panel of serum cytokine and chemokine markers revealed significantly decreased IFN-γ in HNSCC patients[254], which may be caused by inhibition of T-cell regulation from increased expression of PD-1:PD-L1. Immune checkpoint blockade through a monoclonal antibody that inhibits the PD-1 receptor has the potential to play a big role in future therapy, because initiation of anti-tumour response is observed on PD-1 blockade in animal studies[255].

Personalised therapy may be possible with robust biomarker panels and it is detailed molecular analysis, such as DNA profiling[204], that may guide biomarker development. Limited success of individual markers to predict tumour behaviour has led to attempts to classify biomarker "signatures" such as panels of RNA or protein expression alterations[256]. It is possible that miRNA panels associated with HNSCC subsets may also act as biomarkers to improve diagnosis and management[257]. Some studies have investigated panels of predictive biomarkers in both HPV-related oropharyngeal SCC[258] and non-HPV related oropharyngeal SCC[259], however few of these are validated[239].

Research on biomarkers in HNSCC is a rapidly expanding field, with new potential markers that may provide valid therapeutic targets[260], however it is difficult to demonstrate clinical utility without well designed biomarkers or panels undergoing rigorous assessment in clinical trials. Hence many questions remain, with HPV infection as yet not formally validated as a predictive biomarker for any specific treatment modality or agent[256]. More practical diagnostics could be achieved through serum[137,239] or radiological[256] biomarkers, however clinical utility of these remains to be proven. There is no standardisation of detection and when p16 expression is used as a marker for HPV infection, approximately 10% of cases may be false positives[167], such that a combination of p16 overexpression with HPV DNA positivity may currently

represent the most practical investigation for biologically relevant HPV infection[200] and this has been shown to be the most relevant group in terms of prognosis[261]. The relevance of infection in head and neck cancer outside the oropharynx is unestablished and identification of robust fingerprints of HPV carcinogenesis will help to improve the estimate of HPV-related non-oropharyngeal HNSCC.

CLINICAL IMPLICATIONS

HNSCC has a huge impact upon quality of life and longevity. Improvements in clinical outcome have been forthcoming through advancements in surgical technique, radiation oncology and emerging chemotherapeutic and biologic agents, however, despite a multidisciplinary team approach, treatments remain complex with an associated high morbidity and only two new treatments (EGFR antibodies and robotic surgery) have been approved in the past 30 years[262].

HPV-related oropharyngeal SCC, distinct from other HNSCC[39,263], generally presents with a more advanced clinical stage, with a higher nodal category[248,264], despite lower tumour extent[133,264] and have different tendencies for extracapsular spread and perineural invasion[265]. These HPV-related tumours may even be clinically occult, but often present with early lymph node metastases[14,266], which can be confused with branchial cleft cysts[267]. However, tonsil SCCs are long known to present with early lymph node metastases[268] and it may be that the characteristics of the affected site itself facilitate early spread or else potentially the depth of invasion[266].

As stated above, these patients tend to be younger and are less likely to have significant exposure to tobacco and alcohol. Despite more advanced presentation, improved survival, consistently higher than 30%[269], is evident in HPV-related oropharyngeal SCC[66,266,270,271], irrespective of treatment modality[133,220,272-276]. It has been suggested, therefore, that the current classification system for HNSCCs be altered to reflect the different status of HPV-related HNSCCs[273].

Detection of biologically relevant HPV infection is best accomplished using HPV E6 and E7 mRNA, however p16 in combination with HPV DNA correlates well and can be a practical alternative[277]. Studies have also shown an improved response to therapy from HPV-related HNSCCs[16,133,278-280]. As a result of this, it is possible that de-escalation of therapy would be appropriate for these tumours to improve associated morbidity and quality of life. Considering this, there are currently a number ongoing trials. A summary of some of these trials is shown in Table 1.

There have been conflicting reports on the benefit of cetuximab in HPV-related oropharyngeal SCC. While subset analysis in one study suggests improved survival for oropharyngeal SCCs in the cetuximab group (although not necessarily HPV-related)[281], others including the RTOG 0522 and SPECRUM trials disagree[269,282]. Preclinical investigation on treatment effects are limited by the sparse number of HPV-related HNSCC cell lines available.

While organ-preservation trials have led to primary chemoradiotherapy superseding surgical management in HNSCC, there has been renewed interest in transoral techniques for oropharyngeal SCC, particularly with the introduction of robotic surgery. Equivalent early oncologic outcomes to chemoradiotherapy and improved functional outcomes are promising[283]. Some trials involving transoral surgery are shown in Table 1.

Therapeutic vaccines are novel strategies aimed at improving the T-cell mediated immune response to HPV-related SCCs. Recent phase I and Ⅱ clinical trials, some in combination with chemotherapy to boost effectiveness, are investigating these[269,284].

There is currently no single standardized treatment for oropharyngeal SCCs, but before recommended management strategies are altered, results from randomized controlled trials are needed to assess the efficacy of the different treatment modalities available for both HPV-positive and HPV-negative oropharyngeal SCC[285], although recruitment of sufficient numbers remains difficult[265].

Induction of HPV-specific immune responses by prophylactic vaccination with recombinant HPV virus-like particles is likely the key to successful prevention of persistent HPV infection and the subsequent consequences. As such, bivalent and quadrivalent vaccines are now widely available and have shown efficacy in prevention of anal, cervical, vaginal, and vulvar pre-cancers in unexposed individuals[94,286,287]. Unfortunately, present vaccines are only proven to be effective if given before genotype-specific infection is established[288], duration of protection remains unclear and cost is high. Given the high specificity of oropharyngeal cases linked to HPV-16, it is unlikely that other genotypes would replace HPV-16, particularly in view of evidence for induction of cross-genotype immunity with genotype-specific immunisation[289].

In relation to the oropharynx, animal model investigation has revealed reduction in development of HPV-related oral lesions in immunised cases[290]. Recently, an IARC-led study established that a bivalent vaccine used for cervical cancer prevention also reduced oral infections with HPV 16 and 18 by 93.3%[291]. While oral HPV infection is a risk factor for development of HPV-related oropharyngeal SCC, pathogenesis is unclear and the lack of an obvious HPV-related precancerous stage does not facilitate screening and makes evaluation of vaccine effectiveness difficult. Accurate estimates of HPV-related oropharyngeal SCC will help determine the potential role of prophylactic vaccination. It is likely that the effects of vaccination on oropharyngeal SCC will only be revealed over time through longitudinal studies on incidence before and after vaccine introduction.

Treatment of HPV-related oropharyngeal SCC is currently varied geographically depending on tumour stage, patient status including age and co-morbidities, facilities available and HPV or p16 status. There remains uncertainty regarding vaccination, cetuximab and de-escalation

Table 1 Ongoing clinical trials pertaining to treatment of human papillomavirus-related oropharyngeal squamous cell carcinoma

Trial	Phase	Inclusion	Arm 1	Arm 2	Outcomes
RTOG 1016	Ⅲ	p16 positive locally advanced oropharyngeal SCC	Radiation and concurrent chemotherapy	Radiation and concurrent cetuximab	Survival, toxicity, locoregional recurrence and quality of life
ECOG E1308	Ⅱ	Stage Ⅲ-Ⅳa HPV positive oropharyngeal SCC	Complete response to induction chemotherapy and reduced dose radiation with concurrent cetuximab	Incomplete response to induction chemotherapy and standard dose radiation with concurrent cetuximab	Survival, toxicity, response, quality of life and biomarker correlation
De-ESCALaTE HPV	Ⅲ	StageⅢ-Ⅳa HPV positive oropharyngeal SCC	Cetuximab and concurrent radiotherapy	standard concurrent cisplatin and chemoradiotherapy	Morbidity, quality of life, cost, survival and recurrence
QUARTERBACK	Ⅲ	Locally advanced HPV-16 positive oropharyngeal, unknown primary or nasopharyngeal SCC showing complete or partial response to induction therapy	Reduced dose radiation with cetuximab and chemotherapy	Standard dose radiation with chemotherapy	Survival, locoregional control, toxicity and biomarker correlation.
LCCC 1120	Ⅱ	HPV positive and/or p16 positive low-risk oropharyngeal SCC	Decreased dose of radiation and chemotherapy	Standard radiation and chemotherapy	Pathological response rate, locoregional control, survival and quality of life
NCT01221753	Ⅱ	Locally advanced HPV positive oropharyngeal SCC	Docetaxel/cisplatin/5-fluorouracil (TPF) induction chemotherapy followed by concurrent chemoradiation using a modified radiation dose	N/A	Locoregional control, survival and toxicity
SIRS	Ⅱ	Early to mid-stage HPV positive oropharyngeal SCC who receive transoral robotic surgery plus a neck dissection, where clinically indicated	Observation only	Low dose postoperative radiation only Arm 3: Chemoradiation	Rates of locoregional control, overall survival and use of salvage chemoradiation in the observation group
TROG 12.01	Ⅲ	HPV positive oropharyngeal SCC	Radiation and cetuximab	Radiation and cisplatin	Symptoms severity, swallowing, quality of life, toxicity, survival, locoregional recurrence
ADEPT	Ⅲ	p16 positive oropharyngeal SCC that has undergone transoral resection with negative margins	Postoperative radiation alone	Postoperative radiation with cisplatin	Survival, locoregional control, toxicity and quality of life
NCT01088802	Ⅰ / Ⅱ	HPV positive T1-3 oropharyngeal SCC	De-escalated radiation from 70 Gy to 63 Gy with concurrent chemotherapy	De-escalated radiation from 58.1 Gy to 50.75 Gy with concurrent chemotherapy	Toxicity, quality of life and adverse events
ECOG E3311	Ⅱ	Stage III-IVa HPV positive oropharyngeal SCC after transoral surgery and neck dissection with negative margins, no extracapsular spread and less than 4 lymph nodes involved	Transoral surgery with standard radiation	Transoral surgery with low-dose radiation	Survival, surgical complications, toxicity and swallowing

SCC: Squamous cell carcinoma; HPV: Human papillomavirus.

of therapy, which will be made clearer through current prospective trials, leading to better delineation of therapy for HNSCC subsets. Accurate assessment for biologically relevant HPV will be critical to improvement in treatment approaches.

CONCLUSION

HPV has been established beyond doubt as a causative agent in oropharyngeal SCC and biologically active HPV can act as a prognosticator with better overall survival than HPV-negative HNSCCs. A distinct group of younger patients with limited tobacco and alcohol exposure have emerged as characteristic of this HPV-related subset of HNSCC. However, the exact molecular mechanisms of carcinogenesis are not completely described and further studies are needed to assist development of optimal prevention and treatment modalities.

Despite the large pool of research on HPV in HNSCC, great variation exists in detection techniques. Detection of biologically relevant HPV infection will be important for clinical trial design. Also, biomarker discovery will be important not only to identify specific SCC subsets, including those that are HPV-related, to allow for individualised treatment strategies aimed at decreasing morbidity, but also to clarify the role of HPV in non-oropharyngeal sites.

With stored tissue available from the SEER database

in only 271 patients[265], there needs to be greater cooperation between institutions to improve research into understanding this disease. Nevertheless, it is likely that the key approach in future will be prevention and so further studies in prophylactic vaccination, specifically in relation to oropharyngeal SCC, are needed.

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P- Reviewers: Chen GS, Mandic R, Tian YP, Yeudall WA
S- Editor: Wen LL **L- Editor**: A **E- Editor**: Wu HL

World Journal of Clinical Cases

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.12998/wjcc.v2.i6.194

World J Clin Cases 2014 June 16; 2(6): 194-200
ISSN 2307-8960 (online)
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MINIREVIEWS

Therapeutic strategies for targeting the ovarian tumor stroma

Song Yi Ko, Honami Naora

Song Yi Ko, Honami Naora, Department of Molecular and Cellular Oncology, University of Texas MD Anderson Cancer Center, Houston, TX 77030, United States
Author contributions: Ko SY and Naora H contributed equally to this work, generated the figure and wrote the manuscript.
Supported by Cancer and Prevention Research Institute of Texas grant, NO. RP120390 (HN); and United States National Institutes of Health grant, NO. CA141078 (HN)
Correspondence to: Honami Naora, PhD, Department of Molecular and Cellular Oncology, University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 108, Houston, TX 77030, United States. hnaora@mdanderson.org
Telephone: +1-713-5634222 Fax: +1-713-7943270
Received: December 20, 2013 Revised: March 23, 2014
Accepted: May 13, 2014
Published online: June 16, 2014

Abstract

Epithelial ovarian cancer is the most lethal type of gynecologic malignancy. Sixty percent of women who are diagnosed with ovarian cancer present with advanced-stage disease that involves the peritoneal cavity and these patients have a 5-year survival rate of less than 30%. For more than two decades, tumor-debulking surgery followed by platinum-taxane combination chemotherapy has remained the conventional first-line treatment of ovarian cancer. Although the initial response rate is 70%-80%, most patients with advanced-stage ovarian cancer eventually relapse and succumb to recurrent chemoresistant disease. A number of molecular aberrations that drive tumor progression have been identified in ovarian cancer cells and intensive efforts have focused on developing therapeutic agents that target these aberrations. However, increasing evidence indicates that reciprocal interactions between tumor cells and various types of stromal cells also play important roles in driving ovarian tumor progression and that these stromal cells represent attractive therapeutic targets. Unlike tumor cells, stromal cells within the tumor microenvironment are in general genetically stable and are therefore less likely to become resistant to therapy. This concise review discusses the biological significance of the cross-talk between ovarian cancer cells and three major types of stromal cells (endothelial cells, fibroblasts, macrophages) and the development of new-generation therapies that target the ovarian tumor microenvironment.

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Key words: Ovarian cancer; Tumor stroma; Endothelial cells; Fibroblasts; Macrophages; Targeted therapy

Core tip: Despite advances in clinical management, advanced-stage ovarian cancer is still rarely cured by conventional chemotherapy. Substantial efforts have been directed to developing new therapies that target ovarian cancer cells. However, recent studies have revealed important roles of a variety of stromal cells in driving the aggressive behavior of ovarian cancer. Here, we discuss: (1) the significance of three major types of stromal cells in the progression of ovarian cancer; (2) how receptor/ligand-mediated interactions between ovarian cancer cells and stromal cells serve as focal points for therapeutic intervention; and (3) key examples of new-generation agents that target stromal cells.

Ko SY, Naora H. Therapeutic strategies for targeting the ovarian tumor stroma. *World J Clin Cases* 2014; 2(6): 194-200 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i6/194.htm DOI: http://dx.doi.org/10.12998/wjcc.v2.i6.194

INTRODUCTION

Epithelial ovarian cancer is the fifth leading cause of cancer death in women and the most lethal form of gynecologic malignancy[1]. The high morbidity and mortality caused by ovarian cancer primarily stems from late diagnosis. Sixty percent of women who are diagnosed with

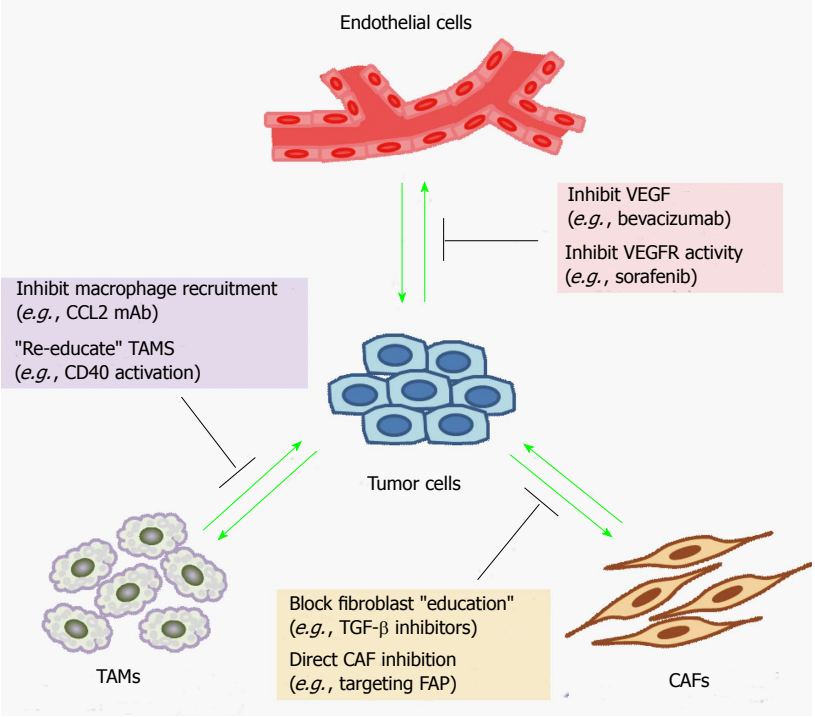

Figure 1 Therapeutic strategies to target the tumor microenvironment. Shown are examples of different strategies and agents that inhibit the regulation of a specific type of stromal cell or its functional properties. Several of these agents are in clinical use, whereas others are at different stages of clinical development. VEGF: Vascular endothelial growth factors; TAMs: Tumor-associated macrophages; CAFs: Cancer-associated fibroblasts; TGF-β: Transforming growth factor-β; FAP: Fibroblast activation protein; CCL2: chemokine (C-C motif) ligand 2; VEGFR: Vascular endothelial growth factor receptor.

ovarian cancer present with extensive peritoneal carcinomatosis and these patients have a 5-year survival rate of less than 30%[1]. For more than 20 years, tumor-debulking surgery followed by platinum-taxane combination chemotherapy has remained the standard first-line treatment[2]. Although the initial response rate is 70%-80%, most patients with advanced-stage ovarian cancer relapse within 18 mo and eventually die from the disease[2]. Substantial efforts have been directed to developing new-generation agents that target functionally relevant molecular aberrations in ovarian cancer cells[3]. Inhibitors of poly (ADP-ribose) polymerase, a DNA repair enzyme, have been undergoing clinical trials in patients with BRCA-deficient ovarian cancer and have attracted considerable attention[4]. In addition to agents that target pathways in ovarian cancer cells, agents that target the tumor vasculature have been the focus of intensive clinical investigation[5,6]. Increasing evidence indicates that ovarian tumor progression is driven not only by dynamic interplay between tumor cells and endothelial cells but also by other types of stromal cells that are "educated" by tumors to acquire properties that are permissive for tumor growth. In this article, we provide an overview of the cross-talk between ovarian cancer cells, endothelial cells and two other key constituents of the tumor microenvironment, specifically, fibroblasts and macrophages, and discuss examples of clinically used and emerging experimental agents that target these stromal cells.

ENDOTHELIAL CELLS

Of the cell types that comprise the ovarian tumor microenvironment, the endothelial cell has been the most extensively studied in terms of its clinical significance. A number of independent studies have identified that increased tumor angiogenesis as manifested by high microvessel density is predictive of poor outcomes in ovarian cancer patients[7-9]. Angiogenesis is a dynamic process that involves the recruitment of endothelial progenitors, growth and maturation of endothelial cells and vessel formation, and is orchestrated by a repertoire of pro-angiogenic and anti-angiogenic factors[10,11]. Key pro-angiogenic factors include the vascular endothelial growth factors (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), angiopoietin, interleukin (IL)-6 and IL-8. Of these factors, VEGF-A has emerged as the predominant pro-angiogenic factor that is highly expressed in ovarian cancers[5,6]. VEGF-A has also been identified to be the causative factor of ascites formation by inducing vascular permeability[12].

Intensive clinical efforts have focused on evaluating agents that inhibit VEGF signaling. These agents fall into two categories: (1) those that inhibit the ligand; and (2) those that inhibit tyrosine kinase activity of the VEGF receptors (VEGFR) (Figure 1). Of the former group, bevacizumab has been the most extensively evaluated agent in ovarian cancer. Bevacizumab is a humanized monoclonal antibody (mAb) that neutralizes all forms of VEGF and was originally Food and Drug Administration-approved in 2004 for treatment of metastatic colorectal cancer. Bevacizumab has been evaluated as a single agent in the treatment of patients with recurrent ovarian cancer in two pivotal phase II trials. In one of these studies (AVF 2949g), the response rate was 15.9% and median overall survival (OS) was 10.7 mo[13]. This study was terminated early due to a high rate of gastrointestinal perforations (5 of 44 patients, 11.4%). In the other study [Gynecologic Oncology Group (GOG) 170D], the response rate was 21.0%, median OS was 16.9 mo, and no bowel perforations were observed[14]. One possible explanation for

the differences in results of these trials is that the GOG study was limited to patients who had received no more than two prior lines of therapy, whereas 21 of the 44 patients in the AVF 2949g study (including the five patients who developed bowel perforations) had received three prior regimens. Bevacizumab has also been evaluated in combination with carboplatin and paclitaxel. In the first-line setting, two phase III trials (GOG 218 and ICON7) reported that progression-free survival (PFS) was increased (by 3.8 and 1.7 mo, respectively) with the combination of bevacizumab and standard chemotherapy followed by bevacizumab maintenance, as compared to standard chemotherapy alone[15,16]. In the recurrent setting, two other phase III studies have found that PFS was increased by approximately 3.6 mo when bevacizumab was combined with standard chemotherapy[17,18]. Another ligand-inhibitory agent is aflibercept, a fusion protein that acts as a soluble VEGFR decoy. In a phase Ⅱ study of aflibercept in patients with recurrent ovarian cancer, the rate of gastrointestinal perforations was found to be low (1.4%) but the primary endpoint of a response rate of greater than 5% was not achieved[19].

Tyrosine kinase inhibitors (TKIs) represent another important class of anti-angiogenic agents. Sorafenib is an oral multi-kinase inhibitor that targets several receptor tyrosine kinases including VEGFR-2, VEGFR-3, platelet-derived growth factor receptor-β (PDGFR-β) and c-kit, and also the RAF family of serine/threonine kinases[20]. In a phase Ⅱ trial of sorafenib monotherapy in patients with recurrent ovarian cancer, two of the 59 evaluable patients had partial responses whereas 20 had stable disease and 30 had progressive disease[21]. Another phase Ⅱ study found that sorafenib did not improve efficacy of first-line carboplatin/paclitaxel treatment and resulted in additional toxicity[22]. Several TKIs that inhibit all three VEGFRs and both PDGFRs have been developed such as sunitinib, cediranib and pazopanib. Sunitinib has been found to have only modest activity as a single agent in patients with recurrent ovarian cancer[23,24]. Clinical trials are ongoing to evaluate cediranib[25] for treatment of recurrent ovarian cancer and pazopanib[26] as maintenance therapy for patients in remission following first-line platinum-taxane chemotherapy.

CANCER-ASSOCIATED FIBROBLASTS

Cancer-associated fibroblasts (CAFs) constitute the cellular fibrotic component of the tumor stroma that is commonly described as "reactive" or desmoplastic stroma. CAFs are often distinguished from normal quiescent fibroblasts by their expression of markers of myofibroblasts and activated fibroblasts such as α-smooth muscle actin (αSMA) and fibroblast activation protein (FAP)[27,28]. CAFs derive from multiple cell types. Two important sources are mesenchymal stem cells (MSCs) and tissue-resident fibroblasts. MSCs are abundant in white adipose tissues such as the omentum[29], the most commonly involved site in ovarian cancer. It has been demonstrated that ovarian cancer cell-derived factors, such as transforming growth factor-β (TGF-β) and lysophosphatidic acid, induce normal omental fibroblasts and adipose MSCs to acquire features of CAFs[30,31]. Studies of other types of tumors have shown that CAFs can also derive from bone marrow MSCs that are recruited to tumors[32,33]. There is evidence in breast cancer that some CAFs derive from tumor cells that have undergone epithelial-to-mesenchymal transition[34]. However, a study of ovarian cancer xenograft models found that stromal αSMA^+ cells did not derive from tumor cells, suggesting that ovarian cancer cells are not a major source of CAFs[31].

Substantial evidence indicates that CAFs contribute to poor survival of cancer patients by promoting tumor cell proliferation, angiogenesis and metastasis[27,28]. In a study of gene expression profiles of clinical specimens of ovarian cancer, Tothill *et al*[35] identified that the subset of cases with the poorest outcomes was characterized by a desmoplastic gene signature. As compared to normal omental fibroblasts, CAFs more highly express IL-6, chemokine (C-X-C motif) ligand 12 (CXCL12) and VEGF-A, and are more effective in stimulating growth of ovarian cancer cells and endothelial cells[31]. The abundance of CAFs in ovarian cancers has been found to correlate with microvessel density[36]. CAFs also highly express TGF-β, matrix metalloproteinases (MMPs) and numerous extracellular matrix proteins[27,28], and stimulate invasiveness of ovarian cancer cells[36]. Furthermore, McLean and colleagues identified that propagating ovarian cancer cells with MSCs derived from ovarian cancer specimens increased the number of cancer stem cells[37]. These findings suggest that another mechanism by which CAFs drive tumorigenesis is by expanding the sub-population of tumor-initiating cells.

Given the profound negative impact of CAFs on outcomes, there have been intensive efforts to develop strategies to target this cell population (Figure 1). Several approaches to inhibit CAFs have been directed to targeting FAP. A humanized mAb to FAP has been found to be well-tolerated, but failed to show efficacy in a clinical trial of patients with metastatic colorectal cancer[38]. In a preclinical study, a DNA vaccine against FAP inhibited tumor growth and increased survival in a mouse colon cancer model[39]. A study by Brennen and colleagues exploited both the expression of FAP on CAFs and its proteolytic activity. These authors generated a prodrug that consisted of a FAP-specific peptide coupled to a thapsigargin analog as the cytotoxic moiety, and demonstrated that the compound induced stromal cell death and inhibited growth of breast and prostate tumor xenografts[40]. Another potential approach to inhibit CAFs is to prevent normal MSCs and fibroblasts from transitioning into CAFs by blocking TGF-β signaling. A number of agents that inhibit TGF-β signaling have been developed including TGF-β-ligand traps, TGF-β antisense oligonucleotides and small molecule inhibitors of the TGF-β type I receptor kinase, and several of these agents have been evaluated in clinical trials[41,42]. The utility of TGF-β inhibitors has been little-explored in ovarian cancer. In one

study, treatment of mice with the TGF-β type I receptor inhibitor A83-01 reduced the fibrotic component of ovarian tumor xenografts but did not increase survival times[43]. Unlike TGF-β, PDGF does not induce myofibroblastic differentiation but instead stimulates fibroblasts to produce mitogenic factors for tumor cells and pro-angiogenic factors. Blockade of PDGFR signaling in a mouse model of cervical cancer has been found to inhibit tumor growth and angiogenesis in part by inhibiting FGF-2 production by CAFs[44]. As discussed earlier, several TKIs that block VEGFR signaling also inhibit the PDGFRs. The impact of these TKIs on the desmoplastic stroma warrants further study as the PDGFRs are often highly expressed in CAFs.

TUMOR-ASSOCIATED MACROPHAGES

Macrophages are normally present in the peritoneal cavity of healthy women and are abundant in ascites of ovarian cancer patients[45]. Tumor-associated macrophages (TAMs) are the major immune component of the tumor stroma[46,47]. Macrophages exhibit polarized phenotypes in response to different microenvironmental cues. Macrophages that are stimulated with microbial agents and interferon-γ exhibit an immunostimulatory M1 phenotype. In contrast, TAMs exhibit an immunosuppressive M2 macrophage phenotype[46,47]. Polarization of macrophages towards an M2 phenotype is induced by stimulation with various cytokines such as IL-6, IL-10 and leukemia inhibitory factor (LIF) that are present at elevated levels in ascites of ovarian cancer patients[48,49]. Chemokine (C-C motif) ligand 2 (CCL2) and TGF-β2 are also expressed in ovarian cancer cells and in CAFs, and these ligands have been recently shown to induce normal peritoneal macrophages to acquire an M2 phenotype[50]. CCL2 is also a key chemotactic factor that is responsible for macrophage infiltration into tumors[47].

TAMS are strongly associated with poor outcomes in cancer patients[46]. A principal mechanism by which TAMs promote tumor progression is by suppressing adaptive immunity. The M2 macrophage phenotype is characterized by high expression of immunosuppressive cytokines and chemokines such as CCL17, CCL18, CCL22, IL-10 and TGF-β1[47]. IL-10 and TGF-β1 inhibit T cell proliferation and dendritic cell maturation[47]. CCL18 induces naïve T cell anergy and has been identified to be the most abundant chemokine present in ovarian cancer patient ascites[51]. CCL17 and CCL22 promote recruitment of T regulatory cells (Treg) cells[52,53]. Treg cells suppress activity of effector T cells and have been found to promote ovarian tumor growth and to be predictive of poor survival in ovarian cancer patients[52]. In addition to expressing factors that suppress adaptive immunity, TAMs express MMPs, VEGF-A and other growth factors that stimulate metastasis and angiogenesis[46,47]. Depletion of peritoneal macrophages has been found to inhibit ascites and peritoneal spread of ovarian cancer in xenograft models[54].

The recruitment of macrophages and their polarization towards a tumor-promoting M2 phenotype represent two candidate focal points for therapeutic intervention (Figure 1). Several approaches have been identified that "re-educate" TAMs towards a more tumoricidal M1 phenotype. Inhibition of the colony stimulating factor-1 receptor has been found to inhibit M2 macrophage polarization and to block glioma progression in animal models[55]. Inhibition of nuclear factor κB signaling in TAMs also induced an M2-to-M1 switch, increased tumoricidal activity of macrophages and led to regression of ovarian tumor xenografts[56]. Activation of CD40, a member of the tumor necrosis factor receptor superfamily, induced tumoricidal activity of macrophages in mouse models of pancreatic adenocarcinoma[57]. The combination of agonistic CD40 mAb and gemcitabine chemotherapy has been found to be well-tolerated and to have anti-tumor activity in a phase I study of patients with advanced pancreatic adenocarcinoma[58]. Zoledronic acid is clinically used to prevent bone fractures and also impairs M2 polarization of macrophages[59]. CCL2 is an attractive target because of its ability to stimulate monocyte chemotaxis as well as M2 polarization. Neutralization of CCL2 induced regression of prostate cancer xenografts[60]. A mAb to CCL2 has recently undergone clinical evaluation[61]. Bindarit, an anti-inflammatory compound that inhibits CCL2 synthesis, has been found to inhibit growth of breast and prostate tumor xenografts[62]. Trabectedin is an alkaloid that binds the minor groove of DNA and disrupts the cell cycle[63]. In a phase Ⅲ study of patients with recurrent ovarian cancer, the combination of Trabectedin and pegylated liposomal doxorubicin (PLD) was found to increase PFS by 1.5 mo as compared to PLD alone[64]. Trabectedin also inhibits production of CCL2 and IL-6 and inhibits the differentiation of monocytes into macrophages[65]. Germano *et al*[66] recently demonstrated the selective toxicity of Trabectedin for macrophages in xenograft models of ovarian cancer and several other solid tumors. In another recent study, Cieslewicz *et al*[67] identified a peptide (M2pep) that selectively binds to M2 macrophages. Administration of a fusion peptide comprising M2pep and a proapoptotic moiety improved survival rates of xenograft-bearing mice[67], raising the possibility that the M2pep peptide could be used as a vehicle for delivering cytotoxic agents to TAMs.

CONCLUSION

Over the past decade, a wealth of insight has been gained into the biology of ovarian cancer, the fertile nature of the peritoneal cavity for carcinomatosis, and the complex networks of receptor/ligand-mediated interactions between tumor cells and stromal cells. Several of the key receptors and ligands serve as molecular targets against which new-generation therapeutic agents have been developed and evaluated. Although several studies have yielded promising results, the efficacy of most stromal-targeting drugs as single agents seems limited. Several challenges remain such as identifying the most effective combinations of these drugs with conventional chemo-

therapy or with other targeted therapies, minimizing toxicity, and determining the appropriate clinical setting for their use.

ACKNOWLEDGMENTS

The authors apologize for the inability to cite all contributing primary literature due to space constraints.

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P- Reviewers: Cavallaro U, Sherbini MAHE **S- Editor**: Ji FF
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World Journal of Clinical Cases

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DOI: 10.12998/wjcc.v2.i6.201

World J Clin Cases 2014 June 16; 2(6): 201-205
ISSN 2307-8960 (online)
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MINIREVIEWS

Concurrent stenoses: A common etiology of stroke in Asians

Bik Ling Man, Yat Pang Fu

Bik Ling Man, Yat Pang Fu, Division of Neurology, Department of Medicine and Geriatrics, Tuen Mun Hospital, Hong Kong, China
Author contributions: Man BL and Fu YP both contributed to this paper.
Correspondence to: Bik Ling Man, MD, Division of Neurology, Department of Medicine and Geriatrics, Tuen Mun Hospital, 23 Tsing Chung Koon Road, Hong Kong, China. manbikling@gmail.com
Telephone: +852-856-24685111 Fax: +852-856-24569111
Received: October 30, 2013 Revised: February 24, 2014
Accepted: March 11, 2014
Published online: June 16, 2014

Abstract

Atherosclerosis of cerebral vessels is a common cause of stroke. Racial differences in the distribution of cerebrovascular occlusive disease are well documented. Extracranial stenosis is more common in Caucasians, while intracranial stenosis is more common in Asians, Hispanics and African-Americans. Concurrent atherosclerosis of extracranial and intracranial vessels is common in Asians. The incidence of concurrent stenoses ranges from 10% to 48% in patients with symptomatic cerebrovascular disease. The long-term prognosis of these patients is poor and they are at high risk of further vascular events or death. The purpose of this review is to examine the epidemiology, risk factors, stroke mechanism and genetics of concurrent stenoses and to discuss strategies for treatment.

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Key words: Atherosclerosis; Concurrent stenosis; Stroke; Asians

Core tip: Concurrent stenoses of extracranial and intracranial vessels are common in Asians, with an incidence that ranges from 10% to 48% in patients with symptomatic cerebrovascular disease. The long-term prognosis of these patients is poor and they are at high risk of further vascular events or death. The purpose of this review is to examine the epidemiology, risk factors, stroke mechanism and genetics of concurrent stenoses and to discuss strategies for treatment.

Man BL, Fu YP. Concurrent stenoses: A common etiology of stroke in Asians. *World J Clin Cases* 2014; 2(6): 201-205 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i6/201.htm DOI: http://dx.doi.org/10.12998/wjcc.v2.i6.201

INTRODUCTION

Cerebrovascular occlusive disease due to atherosclerosis is a common cause of stroke worldwide. However, there are marked racial differences in the distribution of vascular stenosis. Extracranial stenosis is the most common large vessel cause in Caucasians, while intracranial stenosis is more prevalent in Asians, Hispanics and African-Americans[1-4]. Moreover, recent studies suggested that concurrent stenoses of extracranial and intracranial vessels are common in Asians. The purpose of this review is to examine the epidemiology, risk factors, stroke mechanism and genetics of concurrent stenoses and to discuss strategies for investigations and treatment.

EPIDEMIOLOGY

The incidence of concurrent stenoses ranges from 10% to 48% in patients with symptomatic cerebrovascular disease[2,5-8]. Wong *et al*[9] found that 21% of stroke patients had concurrent stenoses in Hong Kong. Yang *et al*[10] report 33% of stroke patients had concurrent stenoses in China. Liu *et al*[8] found that 18% of stroke patients in Taiwan had significant concurrent stenoses. Lee *et al*[6] reported that 48% of patients with more than 30% extracranial carotid stenosis had concurrent intracranial stenoses in South Korea.

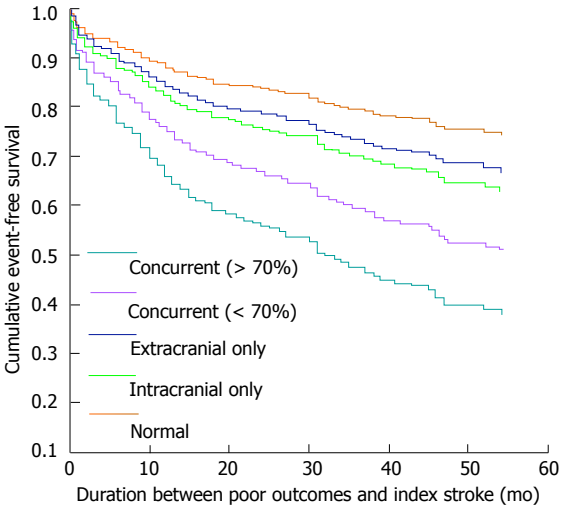

Figure 1 Cumulative event-free survival in patients with different intracranial and extracranial lesions. Concurrent (< 70%): Concurrent lesions with < 70% extracranial stenosis; Concurrent (> 70%): Concurrent lesions with > 70% extracranial stenosis; Extracranial only: Extracranial stenosis only; Intracranial only: Intracranial stenosis only; Normal: Normal craniocervical vasculature.

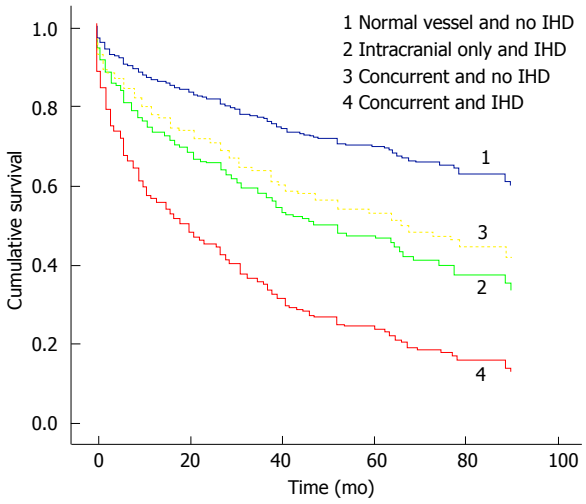

Figure 2 Cumulative event-free survival of combined poor outcomes of different groups of patients. Normal vessel: Normal craniocervical vasculature; Intracranial only: Intracranial stenosis only; Concurrent: Concurrent stenoses.

NATURAL HISTORY OF CONCURRENT STENOSES

The long-term prognosis of ischemic stroke patients with concurrent atherosclerosis of intracranial and extracranial vessels is poor and they are at high risk of further vascular events or death. Our previous studies showed the 5-year cumulative rates of mortality, re-stroke and poor outcomes were 31%, 41% and 51%, respectively (Figure 1)[11]. Furthermore, ischemic stroke patients with concurrent stenoses and ischemic heart disease have an even worse prognosis. The 5-year cumulative rates of mortality, recurrent vascular events and combined poor outcomes were 40%, 50% and 83%, respectively (Figure 2)[12]. On the other hand, patients with concurrent stenoses and small vessel disease have poorer cognitive and functional outcomes[13]. This may be related to the burden of atherosclerosis and synergistic effect of multiple vascular lesions.

RISK FACTORS ASSOCIATED WITH CONCURRENT STENOSES

Our previous studies showed that hypertension[14], diabetes mellitus[15], hyperlipidemia[15], recurrent stroke and poor pre-stroke modified Rankin scale[16] are associated with concurrent stenoses.

ETIOLOGIES

The major cause of concurrent stenoses is atherosclerosis of the cerebrovascular circulation which typically affects large or medium sized arteries. These vessels range from 200-850 μm in diameter and are characterized by the accumulation of subintimal foam cells[16]. In the carotid artery, high risk plaques tend to be severely stenotic[17]. However, severe stenosis of the carotid artery is rare in Asian patients with concurrent stenosis[11]. Compared with extracranial vessels, the adventitia and the media of the intracranial arteries are thinner and their internal elastic lamina are fenestrated differently and thicker[18]. Luminal stenosis, lipid area, presence of neovasculature and inflammatory cells are all associated with ischemic stroke in the middle cerebral artery (MCA) territory[19].

STROKE MECHANISMS

Patients with concurrent stenoses have more symptomatic stenoses, more concomitant perforating artery infarcts, pial infarcts, border zone infarcts and more multiple embolic infarcts in the territory of the leptomeningeal branches of MCA (Figure 3)[14]. The topographic patterns suggest that the combination of hemodynamic compromise attributable to concurrent stenoses and artery-to-artery embolization is a common stroke mechanism in these patients[14].

GENETICS

Genetic factors may play a role in the development of concurrent stenoses on top of the other well established vascular risk factors. Our study showed that genetic polymorphisms of the pathways affecting lipid metabolism and homocysteine are associated with concurrent stenosis[15].

TREATMENT OF CONCURRENT STENOSES

Patients with concurrent stenoses are at high risk of further vascular events or death. The optimal treatment for these groups of patients is still unknown. The American Heart Association/American Stroke Association recom-

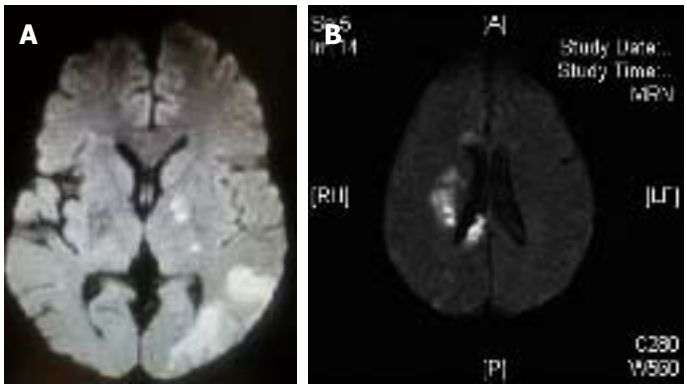

Figure 3 Magnetic resonance imaging diffusion-weighted images of different lesion patterns. A: Concomitant perforating artery infarct and pial infarcts; B: Border zone infarcts.

mend aspirin monotherapy, aspirin/extended-release dipyridamole combination and clopidogrel monotherapy as the acceptable options for all non-cardioembolic ischemic stroke patients[20]. A South Korean study[21] showed that the progression of intracranial stenosis was significantly less in patients taking cilostazol (a phosphodiesterase inhibitor).

However, medical treatment for patients with concurrent stenoses is often unsatisfactory[11,12] and surgical treatment may be indicated in these patients. Although severe carotid stenosis is rare in Asians, patients with severe carotid stenosis are recommended to have a carotid endarterectomy[17,22]. Carotid stenting is not recommended due to high perioperative risks and death associated with this procedure[23,24].

The Carotid Occlusion Surgery Study trial, which investigated the relationship between cerebral hemodynamics and cognitive function in stroke patients undergoing treatment for unilateral carotid artery occlusion with extracranial-intracranial arterial bypass (EC-IC bypass), was stopped prematurely in 2011 because of slow recruitment and a very low incidence of ipsilateral symptomatic ischemic events in patients assigned to the medical arm[25].

The increasing enthusiasm for intracranial stenting for significant intracranial stenosis was dampened by the significant periprocedural neurological complication rate, estimated at 5.3% to 28%[26-30]. The stenting *vs* aggressive medical management for prevention recurrent stroke in intracranial stenosis (SAMMPRIS) trial[31] has been halted due to the high perioperative risks of stroke and death in the treatment arm. However, the risks may be lower at centers with high volume and more experience in these stenting procedures. Jiang *et al*[32] reported on 100 consecutive patients from a single center with a 99% success rate of stent placement and 5% risk of 30 d perioperative stroke and death. Overall, the current evidence does not support the routine use of intracranial stenting in patients with intracranial stenosis.

TRIALS IN PROGRESS

There are two ongoing randomized treatment trials using cilostazol in patients with intracranial stenosis. The first is the Trial of Cilostazol in Symptomatic Intracranial Arterial Stenosis Ⅱ in Asia, which is a double-blind, randomized trial comparing aspirin (75-150 mg per day) in combination with cilostazol (100 mg twice a day) with a combination of clopidogrel (75 mg per day) in patients with significant MCA or basilar artery stenosis[33]. The second is the open-label trial of Cilostazol-Aspirin Therapy Against Recurrent Stroke With Intracranial Artery Stenosis in Japan, comparing open-label aspirin and cilostazol with aspirin alone in patients with symptomatic 50%-99% stenosis of the supraclinoid internal carotid artery, MCA or basilar artery[34]. These trials may provide important information to optimize medical treatment in patients with intracranial stenosis.

Concerning the surgical treatment for intracranial stenosis, the Japanese EC-IC Bypass Trial (JET study) is in progress to determine the ability of STA-MCA bypass to prevent stroke caused specifically by intracranial stenosis, based on evaluations of hemodynamic ischemia[35,36]. The interim analyses of the JET study suggest that in patients with symptomatic intracranial stenosis and evidence of hemodynamic ischemia, surgical intervention with EC–IC bypass is superior to medical management in terms of stroke prevention. The final results of the trial are pending[36].

The Early Stent-assisted Angioplasty in Symptomatic Intracranial Stenosis (ESASIS) trial[37] aims to study the benefit of stenting in reducing the risk of ipsilateral stroke, similar to the SAMMPRIS trial. The Data Safety Monitoring Board of the ESASIS study reviewed the 30 d safety data (combined stroke and death) of 77 randomized patients and found that the safety data of the stenting arm is reassuring when compared with that of the medical arm and is better than the high event rate of 14% being reported in the stenting arm of the SAMMPRIS study. The ESASIS trial is therefore recommended to continue recruitment but with close monitoring of safety.

CONCLUSION

Concurrent intracranial and extracranial stenoses are common in Asians. The patients have a high risk of death and recurrent vascular events. The risk factors include hypertension, diabetes mellitus, hyperlipidemia, previous history of stroke and poor pre-stroke modified Rankin scale. The combination of hemodynamic compromise

attributable to concurrent stenoses and artery-to-artery embolization is a common stroke mechanism in these patients. Optimal treatment for patients with concurrent stenoses is still unknown and more studies are needed on possible interventions which can improve the prognosis of these patients.

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P- Reviewers: Juttler E, Kobayashi T **S- Editor**: Song XX
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DOI: 10.12998/wjcc.v2.i6.206

World J Clin Cases 2014 June 16; 2(6): 206-208
ISSN 2307-8960 (online)
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CASE REPORT

Case of early right ventricular pacing lead perforation and review of the literature

Gary Nash, John Mark Williams, Rajasekhar Nekkanti, Assad Movahed

Gary Nash, John Mark Williams, Rajasekhar Nekkanti, Assad Movahed, Department of Cardiovascular Sciences, East Carolina University, Brody School of Medicine, East Carolina Heart Institute, Greenville, NC 27858, United States
Author contributions: Nash G, Williams JM, Nekkanti R and Movahed A contributed to the manuscript writing and revision.
Supported by "East Carolina Heart Institute"
Correspondence to: Assad Movahed, MD, Department of Cardiovascular Sciences, East Carolina University, Brody School of Medicine, East Carolina Heart Institute, 115 Heart Drive, Mail Stop 651, Greenville, NC 27834,
United States. movaheda@ecu.edu
Telephone: +1-252-7444400 Fax: +1-252-7447724
Received: November 19, 2013 Revised: March 6, 2014
Accepted: May 8, 2014
Published online: June 16, 2014

Abstract

We report a case of a 77-year-old patient with complete atrioventricular block. She underwent permanent pacemaker implantation complicated by right ventricular lead perforation. This was suspected on transthoracic echocardiogram and confirmed by chest computed tomography. The lead was repositioned in the cardiac electrophysiology lab followed by an uneventful course thereafter.

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Key words: Pacemaker; Lead; Perforation

Core tip: Cardiac perforation should be considered in cases of pacing lead malfunction. Chest computed tomography is helpful in diagnosing lead perforation and can be done without contrast and using a small field of view to diminish the effective radiation dose.

Nash G, Williams JM, Nekkanti R, Movahed A. Case of early right ventricular pacing lead perforation and review of the literature. *World J Clin Cases* 2014; 2(6): 206-208 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i6/206.htm
DOI: http://dx.doi.org/10.12998/wjcc.v2.i6.206

INTRODUCTION

Cardiac perforation is a known complication of lead implantation and should be considered in cases of post operative lead malfunction. We present a case of early lead perforation diagnosed by chest computed tomography (CT).

CASE REPORT

A 77-year-old Caucasian female with a past medical history of hypertension, hyperlipidemia and stage 3 chronic kidney disease was brought to the emergency department from home, after her family noticed her "passing out" while seated in a chair. She was noted to regain consciousness within a few seconds. On initial evaluation, her temperature was 36.6 degrees Celsius, blood pressure was 116/61 mmHg, pulse was 43 bpm and regular, respiratory rate was 12 breaths per minute, oxygen saturation on room air was 99%, physical exam was otherwise unremarkable. A sinus node rate of 88 bpm with 2:1 atrioventricular block (ventricular rate of 44 bpm), right bundle branch block and left anterior fasicular block was noted on a 12 lead electrocardiogram (ECG). Exercise myocardial scintigraphy performed one month prior to admission was normal. She was not taking any medications that could cause iatrogenic bradycardia. Ten seconds of ventricular asystole was noted on inpatient telemetry monitoring prompting insertion of a temporary transvenous pacemaker. Six hours later, a dual chamber permanent pacemaker was implanted in the cardiac electrophysiology lab with good post operative sensing and pacing thresholds in the atrium and ventricle. Twelve hours later she complained of left upper quadrant abdominal pain. Inpatient telemetry demonstrated 2:1 atrioventricular block with loss of ventricular capture at high

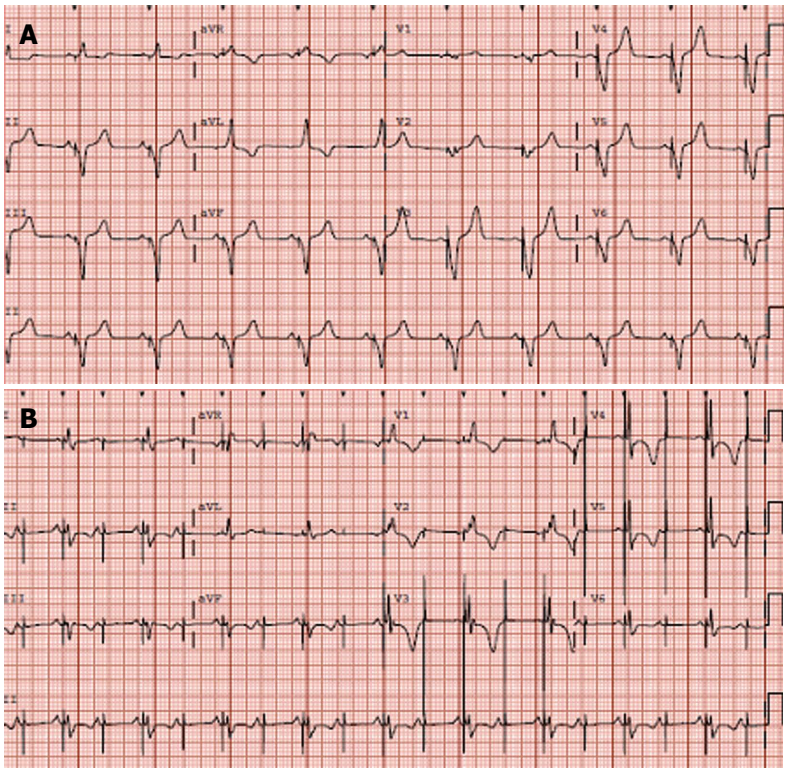

Figure 1 Electrocardiogram. Electrocardiogram on the top panel taken immediately after permanent pacemaker implantation demonstrates atrial sensing with ventricular pacing (A); The electrocardiogram on the bottom panel demonstrates failure to capture in the right ventricle with underlying 2:1 atrioventricular Block (B). The pacing configuration was bipolar with high outputs. Notice the prominent pacing spikes.

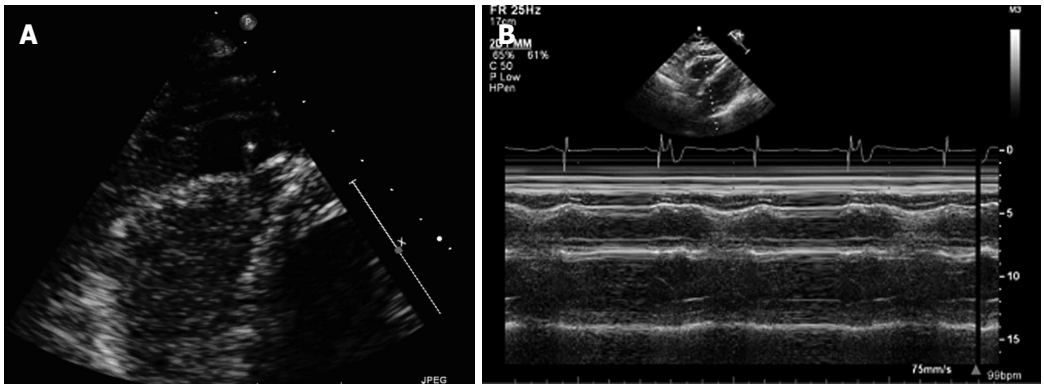

Figure 2 Transthoracic echocardiogram. A: Image is an apical 4 chamber view of a transthoracic echocardiogram showing a moderate sized localized pericardial effusion with an echo bright structure within the effusion suspicious for lead perforation; B: Image is taken in M-mode and demonstrates right ventricular diastolic collapse suggestive of increased pericardial pressures.

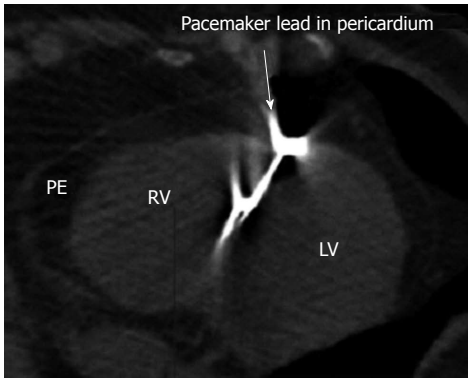

Figure 3 This is an axial view of a non contrast chest computed tomography confirming diagnosis of right ventricular lead perforation. The arrow points to the perforated lead. There is a pericardial effusion (PE). The right ventricle (RV) and left ventricle (LV) are also demonstrated.

pacing output (Figure 1). Chest X-ray did not show any shift in lead positions**.** A temporary transvenous pacemaker was reinserted. Ventricular lead perforation was suspected. A transthoracic echocardiogram demonstrated an echo bright structure protruding into the pericardial space. However, the images were suboptimal in quality and therefore technically limited to confirm lead perforation. A localized moderate sized pericardial effusion with right ventricular diastolic collapse best seen on M-mode imaging (Figure 2) was also noted. She demonstrated no clinical signs of cardiac tamponade. Non contrast chest CT confirmed lead perforation (Figure 3) with the tip of the right ventricular lead in the pericardial space. The lead was repositioned in the cardiac electrophysiology lab under fluoroscopic and echocardiographic guidance (Figure 4). Follow up echocardiogram revealed no change

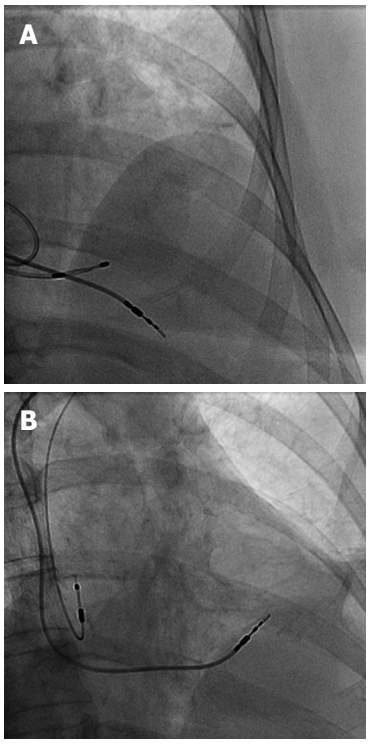

Figure 4 Image. Top image shows the right ventricular permanent pacemaker lead protruding well past the heart border, A temporary transvenous pacemaker lead can also be seen within the right ventricle (A); The bottom image shows the repositioned right ventricular lead higher up on the interventricular septum and absence of the temporary transvenous pacemaker lead. The right atrial lead can also be seen in this image (B).

in the size of the effusion. There was also resolution of right ventricular diastolic collapse. Device interrogation demonstrated stable pacing and sensing thresholds in the right ventricle. The leads remained in stable position on chest X-ray. On follow up 1 wk later the patient was doing well with complete resolution of the pericardial effusion on echocardiogram.

DISCUSSION

Complications associated with permanent pacemaker implantation include pneumothorax, myocardial perforation, lead dislodgement or fracture, infection, hematoma, erosion and vein thrombosis[1]. The rates of cardiac perforation range from 0.1% to 0.8% for pacemaker leads[2]. One should be alerted to the possibility of cardiac perforation by a pacemaker lead if pacing or sensing malfunction is noted. Most cases of lead perforation happen during or shortly after implant, but cases of late perforation as long as 4.8 years after implant have been reported[3]. Chest X-ray has traditionally been used to evaluate lead positioning in cases of suspected pacemaker lead perforation. Echocardiogram can be used to provide additional information such as extent of pericardial effusion. Another option is a non-contrast chest CT utilizing a small field of view to reduce the effective radiation dose. In a small case series Henrikson *et al*[4] demonstrated that 64 slice Chest CT was able to make the diagnosis of cardiac perforation by a device lead in all suspected cases. Risk factors for lead perforation include patient characteristics such as female sex, age, small body habitus, thin heart walls; concomitant therapies such as steroids or anticoagulants; implant techniques; and the design characteristics of the lead[2]. Cardiac perforation by a lead can be corrected by repositioning it under fluoroscopic guidance in the cardiac electrophysiology lab, however surgery may be necessary.

COMMENTS

Case characteristics

This is a 77-year-old Caucasian female with a past medical history of hypertension, hyperlipidemia and stage 3 chronic kidney disease who presented with an episode of syncope.

Clinical diagnosis

She had an episode of ventricular asystole while in the hospital and underwent permanent pacemaker implantation complicated by lead perforation.

Differential diagnosis

Ischemic, infectious, iatrogenic and endocrine causes of atrioventricular block were ruled out.

Laboratory diagnosis

The patient had normal electrolytes, thyroid stimulating hormone level and was not on any atrioventricular nodal blocking agents.

Imaging diagnosis

A non-contrast chest computed tomography confirmed pacemaker lead perforation.

Pathological diagnosis

There were no relevant pathological findings in this case.

Treatment

The patient underwent permanent pacemaker implantation with subsequent lead revision after being diagnosed with cardiac perforation.

Related reports

There are other case reports using various imaging modalities to diagnose cardiac perforation by a pacemaker lead.

Experiences and lessons

Cardiac perforation by a pacemaker lead should be considered in cases of device malfunction regardless of the age of the device.

Peer review

A well done case report. it find no ancillary comments that would aid the readership, continued success with this excellent writing.

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P- Reviewers: Marinkovic SP, Sijens PE **S- Editor**: Qi Y
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DOI: 10.12998/wjcc.v2.i6.209

World J Clin Cases 2014 June 16; 2(6): 209-210
ISSN 2307-8960 (online)
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CASE REPORT

Perforated jejunal ulcer associated with gastric mucosa in a jejunal diverticulum

John Bunni, Helen L Barrett, Tim A Cook

John Bunni, Tim A Cook, Department of Surgery, Gloucestershire Royal Hospital, Gloucester GL1 3NN, United Kingdom
Helen L Barrett, Department of Pathology, Gloucestershire Royal Hospital, Gloucester GL1 3NN, United Kingdom
Author contributions: Bunni J and Cook TA operated on the patient; Barrett HL assessed the pathology; Bunni J conceived and wrote the manuscript; all authors approved it.
Correspondence to: John Bunni, MB, ChB (Hons), MRCS, Registrar in General Surgery, Department of Surgery, Gloucestershire Royal Hospital, Great Western Rd, Gloucester GL1 3NN, United Kingdom. johnbunni@hotmail.com
Telephone: +44-84-54222222 Fax: +44-84-54225612
Received: December 14, 2013 Revised: January 27, 2014
Accepted: May 8, 2014
Published online: June 16, 2014

Abstract

Jejunal diverticula are rare and subsequent complications even more so. The usual small bowel diverticulum encountered by general surgeons is a Meckel's. These are embryological remnants of the vitello-intestinal duct and are on the anti-mesenteric surface of the terminal ileum. They may contain heterotopic gastric or pancreatic mucosa. Herein we explore the case of a young girl who presented with features of peritonitis secondary to a complication from a jejunal diverticulum. The case, pathology, complications and treatment of jejunal diverticulosis and heterotopic gastric mucosa in the jejunum are explored.

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Key words: Jejunum; Perforation; Heterotopic gastric mucosa; Meckel's gastrointestinal diverticulum

Core tip: Herein we describe a rare but important cause of peritonitis in children. We feel it will be of interest to surgeons and pathologists alike and is an important reminder of the basic anatomy and pathology of surgical disease.

Bunni J, Barrett HL, Cook TA. Perforated jejunal ulcer associated with gastric mucosa in a jejunal diverticulum. *World J Clin Cases* 2014; 2(6): 209-210 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i6/209.htm DOI: http://dx.doi.org/10.12998/wjcc.v2.i6.209

INTRODUCTION

Jejunal diverticula are rare and subsequent complications even more so[1]. The usual small bowel diverticulum encountered by general surgeons is a Meckel's. These are embryological remnants of the vitello-intestinal duct and are on the anti-mesenteric surface of the terminal ileum. They may contain heterotopic gastric or pancreatic mucosa.

Complications of true jejunal diverticula are rare particularly in young children. Herein we explore the case of a young girl who presented with features of peritonitis secondary to a complication from a jejunal diverticulum. Following surgery she made an excellent recovery. We explore the case, pathology, complications and treatment of jejunal diverticulosis and heterotopic gastric mucosa in the jejunum.

CASE REPORT

A previously fit and well 5 years old girl presented to the pediatricians with a two day history of worsening abdominal pain, anorexia and vomiting.

Clinical findings were of pyrexia and generalised peritonitis. Bloods tests showed a lymphocytosis of 29.2 × 10^9/L.

A working differential diagnosis was of acute perforated appendicitis or perforated Meckel's diverticulum and she was taken immediately to the operating theatre.

A 1 cm proximal jejunal perforation was identified on the antimesenteric border, directly opposite a mesenteric diverticulum, with a normal appendix. Small bowel re-

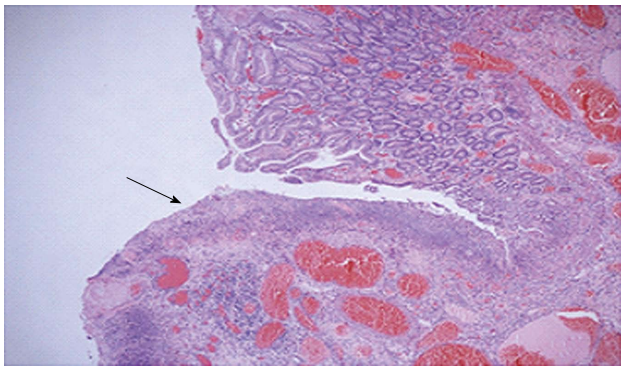

Figure 1 Ulcerated small bowel mucosa (arrow) adjacent to area of perforation.

section was performed with peritoneal toilet. She made a steady recovery and was discharged home on day four post-operatively.

DISCUSSION

Histology of the small bowel showed features of a true diverticulum with gastric mucosa lining the diverticulum. Helicobacter-like organisms were not identified. The adjacent small bowel showed transmural inflammation and degeneration of the wall at the site of perforation with an adjacent serosal exudate (Figure 1). There was no evidence of malignancy. Sections of the appendix showed a mild serosal exudate but no evidence of mucosal acute inflammation.

Jejunal diverticula, like all diverticula can be true, involving all three layers of the bowel wall or false - a herniation of mucosa and submucosa through the muscularis propria. True diverticula are usually congenital and false are acquired. The latter are more common and are pulsion diverticula which arise at the weak point on the jejunal mesenteric surface (the point where the mural vessels penetrate the bowel wall). They are more common in middle aged males.

Rarer are the true diverticula which are congenital in nature. These can be on either the mesenteric or antimesenteric surface. The mesenteric diverticula are thought to be primarily related to the neurenteric remnants and duplications, and can be lined by gastric; intestinal or respiratory type epithelium or contain heterotopic pancreatic tissue.

Complications from jejunal diverticula are varied. Though the majority are silent, they can present with chronic abdominal pain, malabsorption, acute gastrointestinal haemorrhage, occult bleeding, diverticulitis, perforation, bacterial overgrowth or small bowel obstruction due to jejunal volvulus. Complications warranting surgical intervention occur in 8% to 30% of patients[2].

In the elective setting, if suspicious of jejunal diverticulosis there are different investigations that can be performed. These include small bowel follow through, small bowel enteroclysis, capsule endoscopy, computed tomography scanning as well as in the case of bleeding, radionuclide scans and mesenteric angiography.

Jejunal heterotopic gastric mucosa is a very rare entity, and literature review revealed the usual presenting age to be 14 years[3]. A common presentation is that of intermittent intussusception secondary to a small bowel (usually) polypoidal mass. This mass is predominantly located only a few centimetres distal to ligament of Treitz.

Management of jejunal diverticula depends on whether or not patients experience symptoms. Asymptomatic jejunal diverticula found incidentally rarely warrant treatment. In symptomatic cases small bowel resection and end-to-end anastomosis is advised.

Jejunal diverticula are rare and their complications even more so. There are subtle pathological differences and once responsible for complications the authors advise small bowel resection and end-to-end anastomosis so as to achieve a pathological diagnosis; cure the problem and ensure no remaining potential heterotopic mucosa of other organs remains.

COMMENTS

Case characteristics

This young patient presented with an acute abdomen and shock.

Clinical diagnosis

Diagnose with peritonitis. Lab tests showed a lymphocytosis.

Differential diagnosis

Acute appendicitis or perforated Meckel's diverticulum.

Laboratory diagnosis

She was taken to the operating room whereby a small bowel perforation was identified and resected.

Pathological diagnosis

Histopathological analysis revealed this to be a perforation secondary to heterotopic gastric mucosa.

Treatment

Small bowel resection was performed with peritoneal toilet and making a steady recovery.

Experiences and lessons

To rapidly identify the sick patient and not waste time on investigations that will not alter management. Awareness of the diversity of small bowel pathology.

Peer review

This is a short case report showing a pediatric patient with perforating jejunal diverticulum. The case is rare and may be worth publication.

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P- Reviewers: Kim BW, Kato J **S- Editor**: Ma YJ
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World J Clin Cases 2014 June 16; 2(6): 211-214
ISSN 2307-8960 (online)
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CASE REPORT

Abnormal electrocardiogram in a patient with amyotrophic lateral sclerosis mimicking myocardial ischaemia

Juana Martínez, César Ramón, César Morís, Julio Pascual, Germán Morís

Juana Martínez, César Ramón, Julio Pascual, Germán Morís, Neurology Department, Hospital Universitario Central Asturias, 33006 Oviedo, Spain
César Morís, Cardiology Department, Hospital Universitario Central Asturias, 33006 Oviedo, Spain
Author contributions: Martinez J and Morís G contributed to conception and design case history, data, medical care of the patient, drafting case history and revising article for intellectual content; Ramón C and Pascual J contributed to conception and design case history, revising article for intellectual content; Morís C contributed to collection of cardiological data, revision of the manuscript for intellectual content; all authors contributed to final approval of the version to be published.
Correspondence to: Germán Morís, MD, Neurology Department, Hospital Universitario Central Asturias, HUCA. C/Celestino Villamil s/n, 33006 Oviedo, Spain. gmorist@gmail.com
Telephone: +34-98-5108000 Fax: +34-98-5109479
Received: January 5, 2014 Revised: March 26, 2014
Accepted: May 8, 2014
Published online: June 16, 2014

Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that almost exclusively involves motor neurons although autonomic dysfunction has also been reported. We present an 84-year-old female with no documented history of heart disease, who was admitted with negative T waves in the electrocardiogram precordial leads mimicking myocardial ischaemia. No other abnormalities were shown in the rest of the cardiologic evaluation, suggesting autonomic nervous system dysfunction. A neurophysiological study demonstrated acute and chronic denervation in multiple muscles with normal nerve conduction studies, confirming ALS diagnosis. Previous studies have shown that subclinical sympathetic hyperfunction and parasympathetic hypofunction might result in cardiovascular dysfunction in ALS patients. It is important to detect disturbances of autonomic cardiac control because this dysfunction may influence survival and quality of life, leading to a decrease in life expectancy in ALS patients. This Case Report may support the impairment of cardiac autonomic control in patients with ALS.

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Key words: Amyotrophic lateral sclerosis; Autonomic nervous system diseases; Electrocardiography; Myocardial ischemia; Cardiac catheterization

Core tip: A few cases showing electrocardiogram (ECG) abnormalities in amyotrophic lateral sclerosis (ALS) patients have been previously reported suggesting an autonomic disturbance in ALS. We present an ALS patient with abnormal ECG mimicking myocardial ischaemia, in whom both coronary disease and cardiac anatomic damage were ruled out supporting the autonomic nervous system involvement in this mainly motor neuron disease.

Martínez J, Ramón C, Morís C, Pascual J, Morís G. Abnormal electrocardiogram in a patient with amyotrophic lateral sclerosis mimicking myocardial ischaemia. *World J Clin Cases* 2014; 2(6): 211-214 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i6/211.htm DOI: http://dx.doi.org/10.12998/wjcc.v2.i6.211

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an idiopathic, fatal neurodegenerative disease caused by degeneration of the first and second motoneurons. Recent advances indicate heterogeneity in phenotype, pathological substrate and genetic predisposition, suggesting that ALS should be considered a syndrome rather than a single disease entity. Therefore, the clinical presentation and progression of ALS may vary considerably. Cognitive and behavioural impairment is a frequent feature of ALS but other non-motor clinical features, such as autonomic nervous system (ANS) dysfunction, are underreported[1,2]. The

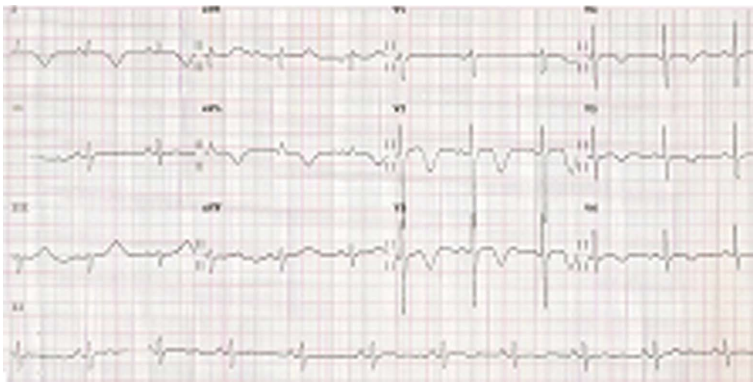

Figure 1 Resting 12-lead electrocardiogram showing negative T waves in precordial leads Ⅰ, aVL, V2-V6.

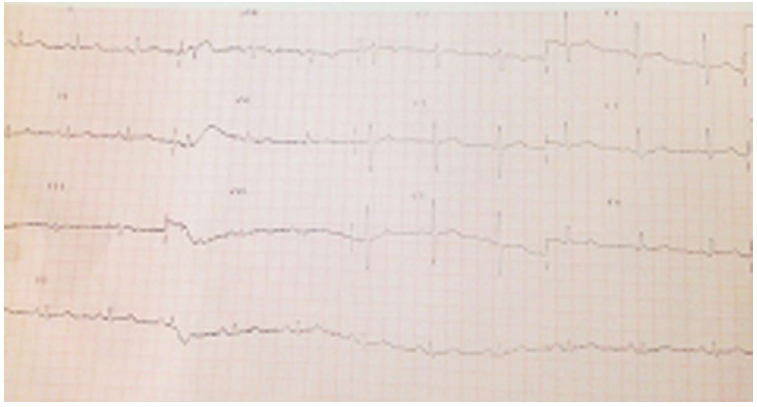

Figure 2 Normal 12-lead electrocardiogram performed in 2002.

affection of the ANS in ALS, as part of a complex degenerative process, has an increasing evidence and it is postulated that ALS patients develop dysautonomic dysfunction that may involve the heart[3].

Herein, we report an ALS patient with both negative T waves on the electrocardiogram (ECG) and no data of underlying coronary disease, supporting ANS dysfunction in ALS.

CASE REPORT

An 84-year-old female with no history of heart disease was referred to our hospital because of chest pain, dyspnoea and abnormal ECG showing negative T waves in precordial leads V2-V6 and Ⅰ, aVL (Figure 1). Initially, the patient had been admitted to a different hospital due to chest pain and progressive dyspnoea. While performing an electromyogram, an episode of shortness of breath, rales on auscultation and desaturation was documented. A chest radiograph was performed suggesting cardiac failure and an ECG showed anterior and lateral subepicardial ischaemia. Diagnostic of acute coronary syndrome was done and the patient was transferred to our Hospital.

The patient had a diagnosis of hypertension made several years before, being under enalapril treatment since then. No other treatments had ever been prescribed. There was no history of myocardial infarction, myocarditis, cardiomyopathy, pericarditis, hyper- or hypothyroidism or calcium metabolism disturbances. An ECG performed 10 years ago showed no abnormalities (Figure 2); no other ECGs were performed before this episode. The patient was feeling well until six months ago, when she started experiencing painless and progressive weakness in her left hand. During the following weeks, weakness progressed to proximal and distal muscles in both upper limbs.

Neurological examination showed a normal mental status. Her speech was dysarthric, no facial paresis was noted but mild lingual weakness was observed. The visual fields were intact and ocular movements were full and smooth. Asymmetric muscle weakness and atrophy, involving the upper extremities, were present with severe atrophy of the left hand muscles. Mild proximal paresis was observed in both lower limbs. Fasciculations were noted in the tongue, upper, and lower extremities. Deep-tendon reflexes were brisk and there were bilateral ankle clonus. There was jaw hyperreflexia. Plantar responses were extensor. There were no sensory deficits. Bowel and bladder function remained normal. Complete blood cell count was normal, as were electrolyte, Troponin T, creatine kinase, creatinine, fasting plasma glucose and haemoglobin A1c concentrations. Liver function and thyroid function studies yielded normal results. A brain CT scan depicted no abnormalities and a cervical MRI showed no abnormal findings at the spinal cord or the nerve roots. A neurophysiological study demonstrated acute and ongoing chronic partial denervation in multiple muscles of bulbar region and both upper and lower extremities with normal nerve conduction studies. The diagnosis of definitive ALS was made according to the Awaji–Shima criteria[4]. Echocardiography showed 16 mm left ventricular

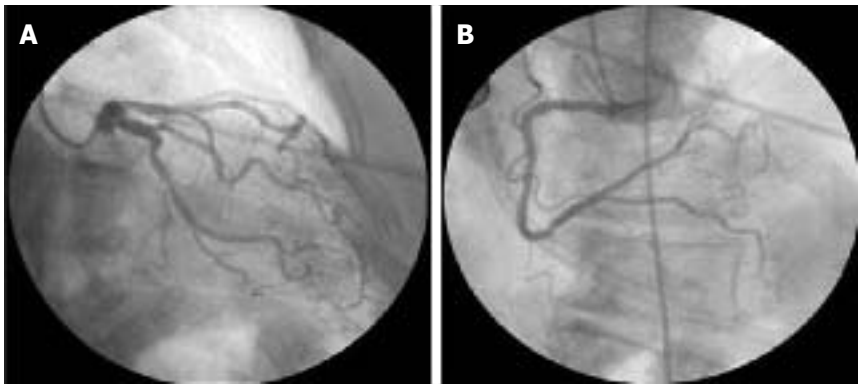

Figure 3 Coronary angiogram showed normal coronary arteries. A: left coronary arteries; B: right coronary artery.

symmetrical hypertrophy with normal wall motion and a coronary angiography showed no significant anomalies (Figure 3). The patient suffered a progressive respiratory failure and she died four days after hospital admission.

DISCUSSION

There is well known that various central nervous system disorders can cause ECG abnormalities that mimic coronary syndromes including S-T elevation, T wave inversion and Q-T prolongation. These findings have been well described in relation with subarachnoid or subdural haematomas. Central o peripheral autonomic dysfunction has been also described in patient with Parkinson´s disease, multiple system atrophy or Guillain-Barré syndrome which increases the risk for arrhythmias, therefore, ECG monitoring is essential[5].

The impact of ALS on the cardiovascular system is well known. Several studies have shown that subclinical sympathetic hyperfunction and parasympathetic hypofunction might result in cardiovascular dysfunction in ALS patients[3,6-10]. There are very few reports describing the ECG characteristics in relation with this pathology. ECG alterations in ALS have been presented as a pseudo-ischaemic pattern, however a pseudo-myocardial infarction pattern has also been described[11-13]. Moreover, an elevated Troponin T levels in a patient with ALS without underlying ischaemic cardiopathy has been reported as a consequence of hypoxic respiratory failure or as immune-mediated myocardial injury secondary to ALS[14].

In ALS, involvement of sympathetic neurons has been associated with neuron degeneration in the intermediolateral nucleus of the upper thoracic spinal cord, causing subclinical findings such as reduction in nocturnal blood pressure and loss of correlation between blood pressure and heart rate. In a study analyzing changes in the corrected QT interval (QTc) and QTc dispersion, ECG showed that both the average QTc and QTc dispersion was significantly higher in patients with ALS supporting sympathetic disturbances in this motoneuron disease[6]. Furthermore, Pavlovic *et al*[3], studied the cardiovascular autonomic control in 55 patients with ALS and compared it with 30 healthy controls. They found that patients with ALS have a significantly higher degree of both sympathetic and parasympathetic dysfunction with relative sympathetic predominance compared with controls. Disturbances of autonomic cardiac control in ALS patients may influence survival and quality of life predisposing to hypertensive crisis, sudden cardiac death, and cardiovascular collapse, all leading to a decrease in life expectancy[3,10,15]. In addition, recent studies have established the contribution of neuronal ion channel dysfunction to the pathophysiology of ALS, mainly Na^+ and K^+ channels; moreover, the modulation of ion channel function has been proposed as the mechanism by which riluzole exerts the neuroprotective effects in ALS[16-18]. Based on ion channels dysfunction implicated in heart disease, it could also be argued that ECG changes in ALS patients may be related with ion channel dysfunction[19].

We present an ALS patient with an ECG showing negative T waves in precordial leads mimicking myocardial ischaemia. Complementary tests ruled out systemic and cardiologic causes that might have been associated with these ECG disturbances; therefore, an association between the pseudo-ischaemic ECG and ALS was suspected. The underlying mechanism of these abnormalities in the ECG must be addressed although ANS dysfunction has been proposed. Unfortunately, neither a baseline ECG nor a follow-up ECG after the acute episode were performed and the only previous ECG was done 10 years before. However, we cannot completely rule out that other underlying processes not covered by our investigations were the cause. In conclusion, it is important to detect disturbances of autonomic cardiac dysfunction in ALS patients to avoid sudden death or other conditions leading to a decrease in life expectancy.

COMMENTS

Case characteristics

An 84-year-old female with a diagnosis of amyotrophic lateral sclerosis (ALS) presented with negative T waves in the electrocardiogram (ECG) mimicking myocardial ischaemia.

Clinical diagnosis

The patient exhibited classical clinical features of ALS and an ECG showed negative T waves in precordial leads I, aVL and V2-V6.

Differential diagnosis

Differential diagnosis included myocardial infarction or ischaemia, myocarditis, cardiomyopathy, pericarditis, hyper- or hypothyroidism or calcium metabolism disturbances.

Laboratory diagnosis

A neurophysiological study demonstrated acute and ongoing chronic partial denervation in multiple muscles of bulbar region and both upper and lower ex-

tremities with normal nerve conduction studies.

Imaging diagnosis

The cardiologic studies including echocardiogram and coronary angiography were normal.

Treatment

The patient did not receive any treatment and she died in a few days.

Related reports

A literature search revealed only a few cases of abnormal ECG in patients with ALS mimicking myocardial ischaemia or infarct.

Term explanation

The Awaji-Shima criteria in the diagnosis of ALS were proposed in 2008 to enable earlier diagnosis of ALS to obviate diagnostic delay and to promote earlier entry into clinical trials.

Experiences and lessons

The affection of the autonomic nervous system in ALS has increasing evidence and it is postulated that ALS patients develop dysautonomic dysfunction that may involve the heart

Peer review

This is a well-written, interesting case report with good images.

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P- Reviewers: Chu D, Hirose H, Riva N, Schoenhagen P, Trohman RG **S- Editor**: Song XX **L- Editor**: A **E- Editor**: Wu HL

World Journal of Clinical Cases

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.12998/wjcc.v2.i6.215

World J Clin Cases 2014 June 16; 2(6): 215-218
ISSN 2307-8960 (online)
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CASE REPORT

Pyonephrosis as a sign of sarcomatoid carcinoma of the renal pelvis

Sergio Fernández-Pello, Victoria Venta, Iván González, Rodrigo Gil, Carmen Luz Menéndez

Sergio Fernández-Pello, Iván González, Rodrigo Gil, Department of Urology, Cabueñes Hospital, 33394 Gijón, Spain
Victoria Venta, Carmen Luz Menéndez, Department of Pathology, Cabueñes Hospital, 33394 Gijón, Spain
Author contributions: All the authors equally contributed to this paper.
Correspondence to: Sergio Fernández-Pello, MD, Department of Urology, Cabueñes Hospital, Calle de los prados 395, 33394 Gijón, Spain. spello84@hotmail.com
Telephone: +43-512-50423401 Fax: +43-512-50424052
Received: December 13, 2013 Revised: January 6, 2014
Accepted: May 8, 2014
Published online: June 16, 2014

Abstract

We report the case of an urgent nephrectomy because of a pyonephrosis and sepsis due to an unsuspected sarcomatoid transitional cell carcinoma, an infrequent subtype with a bad oncological prognosis. We present a 58-year-old man assessed by internal medicine for a general syndrome and weakness many months previously. A pyonephrotic kidney was observed at abdominal computed tomography in the context of septic shock, without suspecting the underlying cause. The pathology report described a sarcomatoid transitional cell carcinoma. Sarcomatoid transitional cell carcinoma is an invasive and infrequent subtype of urothelial tumors. The symptoms are often the same as other renal masses; however, in this case, sepsis and pyonephrosis were the rare initial symptoms.

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Key words: Urothelial carcinoma; Renal pelvis; Sarcomatoid

Core tip: Sarcomatoid transitional cell carcinoma is an invasive and rare subtype of urothelial tumors. The symptoms are often the same as other renal masses; however, in this case, sepsis and pyonephrosis were the rare initial symptoms.

Fernández-Pello S, Venta V, González I, Gil R, Menéndez CL. Pyonephrosis as a sign of sarcomatoid carcinoma of the renal pelvis. *World J Clin Cases* 2014; 2(6): 215-218 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i6/215.htm DOI: http://dx.doi.org/10.12998/wjcc.v2.i6.215

INTRODUCTION

Primary tumors of the renal pelvis account for approximately 7% of all renal tumors[1]; most of them are urothelial carcinoma. Urothelium can display a wide range of metaplastic changes and neoplasms arising from this epithelium can show several types of differentiation, especially in high-grade neoplasms. Unlike bladder urothelial carcinomas, the majority of primary urothelial carcinomas of the renal pelvis present with a high histological grade and show a tendency to display unusual morphological features with a metaplastic phenomena and aggressive behavior. Due to their location inside the kidney, a delay in diagnosis may happen with advanced stages, metastatic disease or massive infiltration of the kidney[2,3]. The sarcomatoid subtype is a kind of high grade urothelial carcinoma, a histological variant defined by a biphasic differentiation, epithelial and mesenchymal. Few cases have been reported in the literature since 1961 when Fauci and collegues reported the first case[4].

CASE REPORT

We present a 58-year-old man assessed for a general syndrome and isolated episode of hematuria a few months previously.

His medical history included being a heavy smoker and alcohol consumer, deep venous thrombosis and alcoholic chronic pancreatitis with partial pancreatectomy 10 years previously.

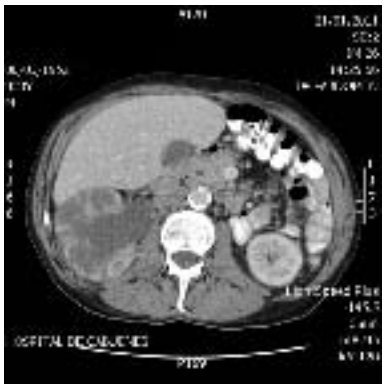

Figure 1 Contrast enhanced abdominal computed tomography shows a dilated and unstructured right kidney. No mass effect images.

Figure 2 Macroscopic sagittal cut of the right kidney after nephrectomy, necrotic areas spread outside kidney and parenchyma is replaced by fibrotic tissue.

From the urological point of view, an isolated episode of gross hematuria was described and was concomitant with a high dosage of oral anticoagulants. At this time, ultrasound and cystoscopy were within the normal limits.

In hospital, he was afebrile with low levels of blood pressure. His blood analysis showed anemia (hemoglobin 8.6 g/dL), discrete leukocytosis and serum creatinine within normal limits.

An abdominal computed tomography (CT) was requested which described a bizarre right kidney with intraparenchymatous levels of liquid with the renal pelvis and right ureter dilated up to the entrance of the bladder (Figure 1).

Initially, endovenous third generation cephalosporin treatment was administered and a ureteral stent was placed. After 12 h of observation, the clinical course worsened, with severe hypotension and elevated blood analysis parameters of lactic acid, C protein and procalcitonin. In this critical situation of septic shock, an emergency right nephrectomy was performed with an eleventh rib incision. The intraoperative description was of a dilated right kidney with plentiful purulent liquid linked with a pyonephrosis with no apparent cause. The patient had a normal postoperative course and was discharged on the seventh day.

The surgery specimen weighed 500 g and the volume was 15 cm × 9 cm × 7 cm, with thick parenchyma with pyelocalyceal dilation with purulent content at the sagittal section. Necrotic areas spread outside the renal tissue, the perinephric fatty tissue (Figure 2).

The pathology report described a sarcomatoid urothelial carcinoma with invasion into the parenchyma, renal sinus, perinephric fatty, lymphovascular and neural tissue. Carcinoma *in situ* was also associated with the upper calyceal system according to the American joint committee of cancer (AJCC), seventh edition, pT4NX. Immunochemistry studies revealed positivity to CK7, CD10 and vimentin. Moreover, the report described intense proliferative activity Ki67, 80% (Figure 3).

Chemotherapy was rejected due to the clinical situation of the patient and the patient refused a new surgical intervention in order to eliminate the ureteral remnant. The patient was followed up periodically and 18 mo after nephrectomy he was asymptomatic with no signs of relapse on imaging techniques.

DISCUSSION

Transitional cell carcinoma of the renal pelvis and ureter are relatively rare and are less than 1% of all genitourinary cancers, likewise 5% and 7% of urinary tract tumors[5]. Tumors arising from the renal pelvis are morphologically similar to those from the bladder. Their incidence varies from 0.7 to 1.1 per 100000 with a male to female ratio of 1.7 to 1, but with an increasing trend in women. They more frequently appear in the elderly (mean age 70 years). Over 90% of tumors arising from the renal pelvis and ureter are urothelial carcinomas. Hematuria and back pain are the most common signs and symptoms[6]. Hematuria, either gross or microscopic, is present in 75%-90% of cases. Back pain occurs in 20%-40% of cases, usually secondary to obstruction by the tumor that can mimic a ureteral calculus. Urinary symptoms, such as dysuria, urinary frequency, nocturia or urinary retention, can be found in up to 25%-50% of patients. The physical examination is usually normal, with the exception of a lumbar palpable mass in less than 10% of patients[5].

Sarcomatoid transitional cell carcinoma (sarcomatoid carcinoma) of the renal pelvis should not be confused with sarcomatoid renal cell carcinoma, an undifferentiated high grade epithelial tumor whose origin is at the parenchyma. Another tumor with a bad prognosis is the collecting duct carcinoma (Bellini duct carcinoma) which is centered in the medulla, develops a tubulopapillary architecture and is surrounded by a desmoplastic reaction[7]. Radiologically, some researchers reported that sarcomatoid carcinomas and renal cell carcinomas are indistinguishable from each other.

The term "transitional cell carcinoma with sarcomatoid differentiation" should be used in solid tumors with biphasic epithelial and mesenchymal differentiation (with the presence or absence of heterologous components).

Another neoplasm considered at the differential diagnosis is carcinosarcoma. Carcinosarcomas and sarcoma-

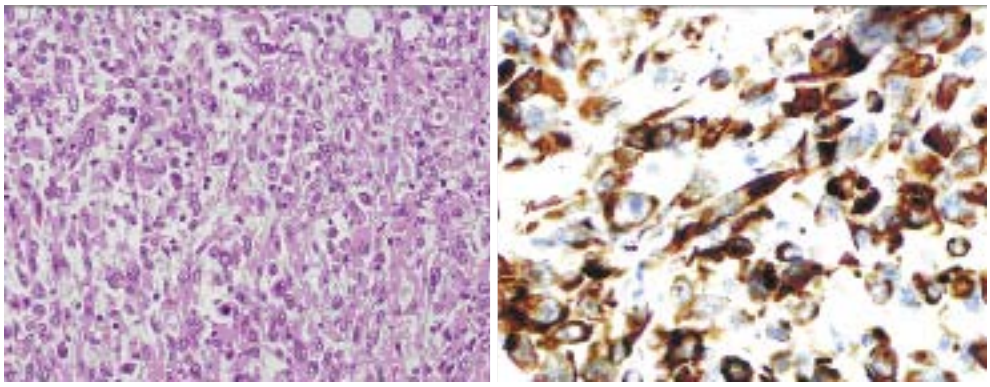

Figure 3 On the left side the sample (x 20) shows the invasive sarcomatoid pattern and on the right side the immunochemistry (x 40) positivity for CK7.

toid carcinomas are difficult to distinguish in hematoxylin-eosin samples. Carcinosarcomas are composed of two components, epithelial and sarcomatous, and sarcomatoid carcinomas are malignant epithelial tumors which show sarcomatoid changes[8]. There is too much confusion and disagreement in the literature regarding the nomenclature and histogenesis of these tumors. In some series, both terms, carcinosarcoma and sarcomatoid carcinoma, are included under the term "sarcomatoid carcinoma" and in others they are listed as two different entities[3,4,9,10].

In addition, the differential diagnosis should also include locally aggressive and benign conditions, such as postoperative spindle cell nodes and pseudotumors as inflammatory myofibroblastic tumors.

Histologically, sarcomatoid areas may be combined with foci of transitional cell carcinoma, squamous cell carcinoma, adenocarcinoma or small cell carcinoma. Heterologous differentiation may be present but has no prognostic significance. All sarcomatoid carcinomas are high-grade and have a poor prognosis. These tumors show no difference in survival when compared stage by stage with conventional urothelial carcinoma. In the absence of invasive urothelial carcinoma or obvious epithelial differentiation, a prior history of urothelial carcinoma, the coexistence of urothelial carcinoma *in situ*, or immunoreactivity for cytokeratin or epithelial membrane antigen (EMA) in the sarcomatous areas are useful for the diagnosis of sarcomatoid carcinoma. The immunohistochemistry is the key to the diagnosis.

The immunoreactivity for keratins and EMA are specific for epithelial cells and the presence of epithelial markers in mesenchymal areas and/or the presence in sarcomatoid elements of ultrastructural features of epithelial differentiation (desmosomes or tonofilaments) suggest the diagnoses of sarcomatoid carcinoma. Likewise, the mesenchymal elements in carcinosarcomas do not stain with epithelial markers and have no desmosomes or tonofilaments[3].

The gold standard treatment for urothelial carcinoma is surgery, in this case nephroureterectomy, but a nephrectomy was only performed because the presence of an aggressive tumour was unsuspected. Neoadjuvant chemotherapy was not an option for this patient. On the one hand, it was an emergency operation and on the other hand, contrary to what has been demonstrated for bladder cancer, there have been no reported effects of neoadjuvant therapy for upper urinary tract cancer[11].

Adjuvant chemotherapy can somehow achieve a recurrence free rate of up to 50% but clearly has no impact on survival and no data are currently available to provide any recommendations[12]. At our institution, chemotherapy is only considered as palliative in cases of metastatic evolution or with the presence of symptoms.

Adjuvant radiotherapy may improve local control of the disease and can be combined with a cisplatinum regimen[13] but they are no longer considered at our center as a standard care for this kind of tumors.

This pT4Nx tumor is included in the IV stage according to AJCC classification (remember IV stage includes all pT4 with N+ or N0 and M+ or M0), the fact of pT4 being directly included in IV prognostic stage in spite of no pathological node report and no signs of metastatic disease. The observed overall survival with this classification with data taken from National Cancer Data Base for the year 2000 to 2002 is: 43.3% in 1 year, 24% in 2 years, 16.4% in 3 years, 12.4% in 4 years and 10.2% in 5 years.

An interesting paper describes the local relapse rate during a mean follow-up of 58 mo in 14% of the patients, with the overall mortality of 14% and the mean survival of 109 mo. Stage T3 and T4 were significantly linked with survival[14].

We report the case of a 58-year-old man with the radiological finding of pyonephrosis and an emergency nephrectomy being performed with the worsening of the clinical condition. The pathology examination suggested a renal pelvis urothelial neoplasm with sarcomatoid differentiation. This neoplasm widely spread though perirenal fatty tissue and lymphatic vascular tissue.

Sarcomatoid subtype is a rare presentation of urothelial carcinoma and is linked with a bad prognosis. In the same sample, zones of epithelial carcinoma, adenocarcinoma and small cell carcinoma are usually described.

COMMENTS

Case characteristics

A 58-year-old man assessed for a general syndrome and isolated episode of hematuria.

Clinical diagnosis
The radiological finding of pyonephrosis and worsening of the clinical condition, with an emergency nephrectomy being performed.

Differential diagnosis
Ultrasound, cystoscopy, computed tomography (CT).

Laboratory diagnosis
Blood analysis showed anemia (hemoglobin 8.6 g/dL). The surgery specimen weighed 500 g and volume was 15 cm × 9 cm × 7 cm, with thick parenchyma with pyelocalyceal dilation with purulent content at the sagittal section.

Imaging diagnosis
An abdominal CT was requested and described a bizarre right kidney with intraparenchymatous levels of liquid, with the renal pelvis and right ureter dilated up to the entrance of the bladder.

Pathological diagnosis
The pathology report described a sarcomatoid urothelial carcinoma with invasion into the parenchyma, renal sinus, perinephric fatty, lymphovascular and neural tissue.

Treatment
The clinical evolution with an emergency nephrectomy.

Experiences and lessons
Sarcomatoid subtype is a rare presentation of urothelial carcinoma and is linked with a bad prognosis. In the same sample, zones of epithelial carcinoma, adenocarcinoma and small cell carcinoma are usually described.

Peer review
The authors describe a case of pyonephrosis as a sign of sarcomatoid carcinoma of the renal pelvis. This is an interesting paper.

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P- Reviewers: Ansari MS, Di Lorenzo G, Maurer T, Papatsoris AG
S- Editor: Ma YJ **L- Editor**: Roemmele A
E- Editor: Wu HL

World Journal of Clinical Cases

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DOI: 10.12998/wjcc.v2.i6.219

World J Clin Cases 2014 June 16; 2(6): 219-223
ISSN 2307-8960 (online)
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CASE REPORT

Two-level reconstruction of isolated fracture of the lesser tuberosity of the humerus

Vasileios S Nikolaou, Dimitrios Chytas, Evangelos Tyrpenou, George C Babis

Vasileios S Nikolaou, Dimitrios Chytas, Evangelos Tyrpenou, George C Babis, 2nd Orthopaedic Department, Athens University, 15124 Athens, Greece
Author contributions: Nikolaou VS performed the surgery and wrote the manuscript; Chytas D and Tyrpenou E did the literature search and prepared the first draft; Babis GC did the final checking and proof editing of the manuscript.
Correspondence to: Vasileios S Nikolaou, MD, PhD, Orthopaedic Surgeon, Lecturer in Orthopaedics, 2nd Orthopaedic Department, Athens University, 21 Dimitriou Ralli Str, Maroussi, 15124 Athens, Greece. vassilios.nikolaou@gmail.com
Telephone: +30-6932-543400 Fax: +30–210-8022142
Received: December 29, 2013 Revised: April 17, 2014
Accepted: May 14, 2014
Published online: June 16, 2014

Abstract

Fractures of the lesser tuberosity of the humerus are typically met in combination with other injuries of the shoulder. Case reports of isolated lesser tuberosity fractures are particularly rare and, consequently, therapeutic protocols have not yet been completely clarified. Conservative as well as surgical treatment has been recommended, while several operative techniques have been applied. We present a case of a 39-year-old man with an isolated lesser tuberosity fracture who was treated surgically in our institution. Due to fracture comminution, a two-level reconstruction technique with headless screws and buttress plate was applied. As far as we know, this method of fixation of this type of fracture has not been previously described in the literature. The patient tolerated the procedure well and excellent results were obtained at the latest follow-up.

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Key words: Shoulder; Humerus; Lesser tuberosity; Fixation; Technique; Two-level reconstruction

Core tip: Isolated fractures of the lesser tuberosity of the humerus in adults are extremely rare. Only a few cases have been reported so far in the literature. The optimal treatment method of these fractures is still a matter of debate. Herein, we present a case of a 39-year-old man with an isolated lesser tuberosity fracture who was treated surgically in our institution. Due to fracture comminution, a two-level reconstruction technique with headless screws and buttress plate was applied. As far as we know, this method of fixation of this type of fracture has not been previously described in the literature.

Nikolaou VS, Chytas D, Tyrpenou E, Babis GC. Two-level reconstruction of isolated fracture of the lesser tuberosity of the humerus. *World J Clin Cases* 2014; 2(6): 219-223 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i6/219.htm DOI: http://dx.doi.org/10.12998/wjcc.v2.i6.219

INTRODUCTION

Five percent of all humeral fractures involve the proximal end alone[1,2]. Out of this, only 2% account for isolated lesser tuberosity fractures, making the incidence extremely rare. More often, they are seen as an isolated injury or with a combined posterior dislocation of the shoulder[3]. Moreover, these fractures may be difficult to identify because of the osseous overlapping in the standard x-ray examination or they are misdiagnosed as intra-articular loose bodies or calcifications of the rotator cuff[4].

The injury typically requires traumatic abduction and external rotation of the upper arm in relation to the shoulder. The forceful contraction of the subscapularis muscle leads to the avulsion fracture of the lesser tuberosity.

Epidemiologically, little is known but it has been described as an injury that involves male patients between the 2nd and 5th decades of life and youngsters with an open humeral physis. If not treated or missed, these injuries may lead to subscapularis weakness and/or impinge-

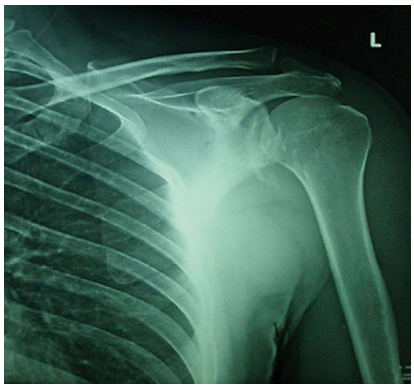

Figure 1 Anteroposterior radiograph of left shoulder showing a crescent-shaped fragment near the inferior part of the glenoid rim.

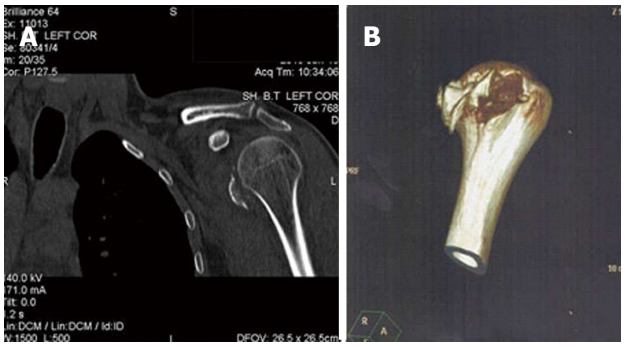

Figure 2 Further examination with computed tomography scan (A) and 3D computed tomography reconstruction (B) confirmed the diagnosis and revealed the comminution of the lesser tuberosity avulsion fracture.

ment of the malunited part[5,6].

Generally, open reduction and internal fixation is the treatment of choice in otherwise medically fit patients but some authors suggest conservative treatment for minimally displaced fractures and report clinically successful results[7,8].

Operative procedures can be challenging in cases of comminuted fractures of the lesser tuberosity. We present a case of a comminuted isolated avulsion fracture of the lesser tuberosity of the humerus in a young male patient. A two-level reconstruction technique is described.

CASE REPORT

A 39-year-old male presented to our emergency department with a history of a fall from a height of about 2 m on to his outstretched left arm. On clinical examination, there was tenderness on palpation on the frontal aspect of the proximal humerus and restriction of movements of the joint, energetically and passively. No neurovascular damage was noted.

Standard anteroposterior radiograph revealed a crescent-shaped fragment near the inferior part of the glenoid rim. At that point, lateral X-ray was not possible due to patient's pain (Figure 1).

The patient was admitted to our orthopedic department and a computed tomography (CT) scan was performed to describe the damage in greater detail (Figure 2). A magnetic resonance imaging (MRI) scan was also performed to exclude possible intra-articular damage and/or tendon pathology (Figure 3).

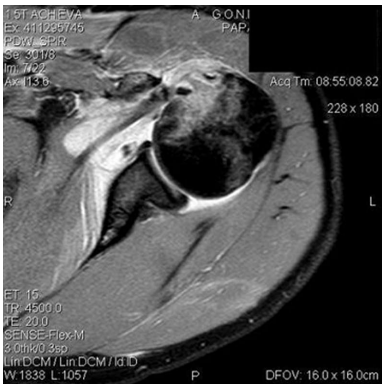

Figure 3 Magnetic resonance imaging exam of the left shoulder excluded intra-articular extension of the fracture or other tendon ruptures.

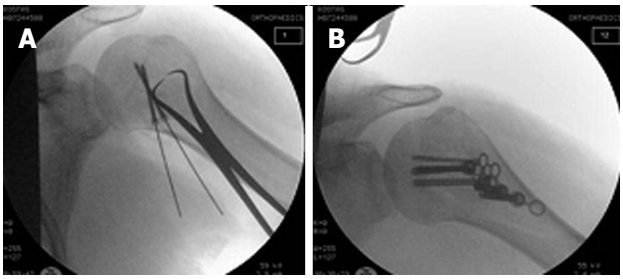

Figure 4 Intra-operative fluoroscopic images. Initially, the bigger fragments of the lesser tuberosity were reduced using reduction forceps and fixed using cannulated headless Hebert screws (A). The smaller fragments were then reduced and stabilized under a low profile, bendable neutralization buttress plate (B).

The isolated comminuted avulsion fracture of the lesser tuberosity was confirmed, with no other intra-articular pathology of the shoulder joint. It was decided to operate and the patient was transferred to the operating room 48 h after the injury.

Under general anesthesia and with the patient supine in the so-called beach chair position, with the image intensifier placed on the opposite side of the operating table, a standard deltopectoral approach was used. Fragments of the avulsed lesser tuberosity with the subscapularis tendon were identified. The fracture did not extend to the articular surface or the bicipital groove. Initially, the larger fragment of the avulsed lesser tuberosity was reduced using reduction forceps. Two cannulated Herbert screws were used to stabilize the fragment (Figure 4). The smaller fragments were then reduced and stabilized using a low profile, bendable neutralization buttress plate (T-plate, NCB proximal humerus, Zimmer Company, Winterthur, Switzerland) (Figure 5). The long head of the biceps tendon was identified and found to be stable (Figure 6). Excellent fracture reduction was confirmed with intraoperative fluoroscopy (Figure 4). A drain was positioned and the wound was closed in the usual fashion. Figure 7 shows the immediate post-operative X-ray.

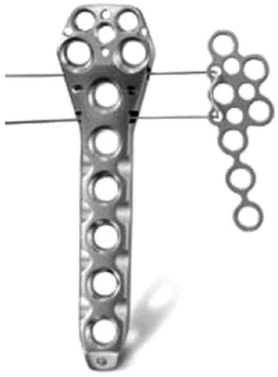

Figure 5 The 7-holes T-minus plate (right) that was used as a buttress plate is part of the Non-Contact Bridging proximal humerus, polyaxial locking plate system (Zimmer Company, Winterthur, Switzerland).

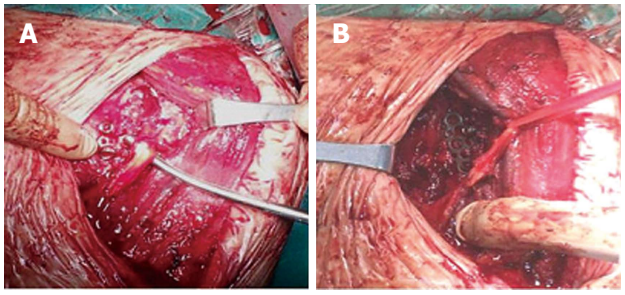

Figure 6 Intraoperative photos. A: The headless Herbert screw is inserted and the larger fragments have been stabilized; B: The T-minus plate has been positioned acting as a buttress plate. The biceps tendon was found to be stable and was protected during the procedure.

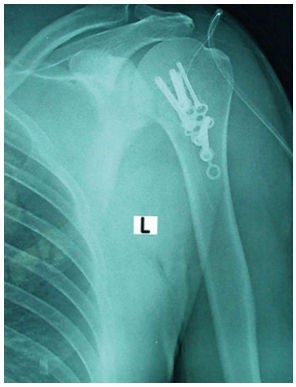

Figure 7 Immediate post-op X-ray of the left shoulder, showing excellent fracture reduction.

The arm was placed in a sling in a neutral position and the elbow flexed 90 degrees. The patient was released from the hospital 2 d post-operatively. Immediately after stitches removal, the patient initiated his rehabilitation program, with passive assisted exercises to regain full range of motion. Six weeks post-operatively, active exercises were implicated.

On follow-up, the patient had achieved painless full range of motion and regained his normal activities. X-ray examination at the latest follow-up, 18 mo after surgery, revealed full union of the fracture and no hardware failure (Figures 8 and 9).

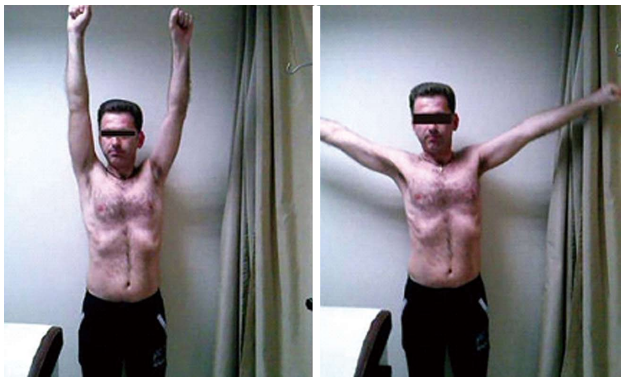

Figure 8 At the latest follow-up, the patient demonstrated pain-free, full range of movement of the left shoulder.

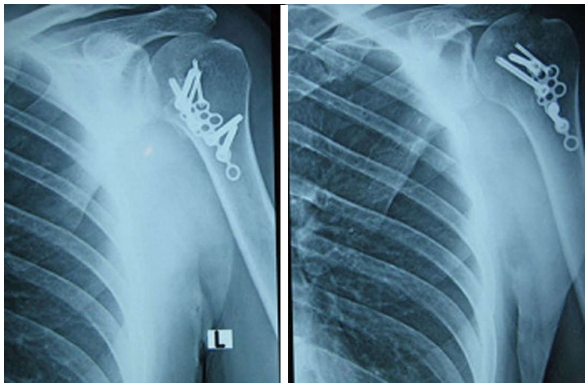

Figure 9 Eighteen months after surgery, X-ray examination of the left shoulder shows full union of fracture without hardware movement or failure.

DISCUSSION

Approximately 2% of proximal humeral fractures are isolated fractures of the lesser tuberosity[9]. This demonstrates how rarely those injuries are met. Until now, the lack of an extensive published case series does not allow the determination of a standard therapeutic protocol for this type of fracture. The small sized lesser tuberosity, which is adequately protected in the medial aspect of the proximal humerus, is not frequently fractured and, when this happens, sudden contraction of the subscapularis muscle, which is attached to the tuberosity and prevents the abduction and external rotation of the shoulder, is the most common mechanism of injury[6]. The clinical presentation of the patient, characterized by pain particularly in the frontal aspect of the shoulder and restricted motion, is not typical and requires proper radiographic control, which generally includes at least two views of the shoulder. Although in our case the fracture can be noted in the anteroposterior view of the shoulder, often the diagnosis is missed or delayed due to the lack of a complete imaging. An axillary view especially generally demonstrates lesser tuberosity fractures most clearly and possibly displaced fragments[10]. However, on plain radiographs, these fractures may be misdiagnosed as calcific tendonitis

of the rotator cuff or osseous Bankart lesions[8].

Thus, the usefulness of a CT scan in the diagnosis and treatment of this type of fracture, including the surgical technique for its fixation, is particularly important[2]. More specifically, a CT scan is a valuable tool in the hands of the surgeon for the estimation of specific characteristics of the fracture, such as displacement, comminution and possible involvement of the articular surface[2]. Further investigation with MRI can also help in determining possible severe soft tissue damage, including rotator cuff tendon injury, or severe trauma of the articular surface.

Once the diagnosis is made, several therapeutic options have been proposed. Conservative treatment, although not frequently indicated, has its own remarkable position in the therapeutic "arsenal", particularly in minimally displaced fractures[10] and in children[11,12]. However, controversy exists about the displacement of fracture as an indication of surgery; some authors recommend open reduction and internal fixation of even minimally displaced fractures in order to avoid late displacement and involvement of the bicipital groove[7]. Generally, operative treatment is recommended in cases of displacement more than 5 mm, angulation more than 45 degrees, persistent pain, blockage to motion and significant clinical weakness[5].

Regardless of the size or displacement of the fractured fragments, a review of the literature demonstrates that open reduction and internal fixation is the gold standard of the management of isolated lesser tuberosity fractures. Apart from open reduction and internal fixation, other methods of surgical treatment do exist and have generally given satisfactory results; surgical excision of the fractured fragment[13-15] and arthroscopically assisted reduction[16] have been proposed by several authors and have been proven efficient.

The common technique of open reduction and internal fixation of isolated lesser tuberosity fractures described in the literature includes the use of screws, cerclage wire[6] and, particularly in skeletally immature patients, the use of heavy sutures and suture anchors[5].

In conclusion, as far as we know, a two-level reconstruction of this type of fracture with headless screws and a buttress plate has not been previously described. The fact that led us to this surgical option was the comminution of the fracture and subsequent inability of adequate fixation with screws only. The larger fragments of the fracture were successfully fixed with headless Herbert screws. The smaller fragments were buttressed using a low profile, multi-hole plate. This plate also provided a more secure fixation of the larger fragments. As was proven, the clinical outcome was satisfactory and comparable with other cases in which different surgical methods were applied.

COMMENTS

Case characteristics

Isolated fracture of the lesser tuberosity of the humerus in a young adult male.

Clinical diagnosis

There was tenderness on palpation on the frontal aspect of the proximal humerus and restriction of movements of the joint, energetically and passively. No neurovascular damage was noted.

Differential diagnosis

Shoulder fracture dislocation, rotator cuff pathology and/or osseous Bankart lesion were considered in the differential diagnosis based on patient's symptoms. X-ray examination, computed tomography (CT) scan with 3D reconstruction and magnetic resonance imaging (MRI) of the injured shoulder were carried out.

Imaging diagnosis

Standard anteroposterior radiograph revealed a crescent-shaped fragment near the inferior part of the glenoid rim. Further examination with CT scan and 3D CT reconstruction confirmed the diagnosis and revealed the comminution of the lesser tuberosity avulsion fracture. MRI exam of the left shoulder excluded intra-articular extension of the fracture or other tendon ruptures.

Treatment

Due to fracture comminution, a two-level reconstruction technique with headless screws and buttress plate was applied.

Experiences and lessons

Isolated fractures of the lesser tuberosity of the humerus are extremely rare in adults. Open reduction and internal fixation is usually the treatment of choice. In the setting of severe fragment comminution, adequate stabilization of the fractured lesser tuberosity can be challenging. Using the proposed operative technique, with two-level reconstruction of the lesser tuberosity, resulted in excellent fracture healing and the clinical outcome was satisfactory and comparable with other cases in which different surgical methods were applied.

Peer review

This is a well written case report describing a new surgical approach. It will be of general interest to orthopedic surgeons.

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P- Reviewers: Naugler C, Serhan H, Solomon LB
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World J Clin Cases 2014 June 16; 2(6): 224-227
ISSN 2307-8960 (online)
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CASE REPORT

Simultaneous bilateral robotic partial nephrectomy: Case report and critical evaluation of the technique

Claudio Giberti, Fabrizio Gallo, Maurizio Schenone, Pierluigi Cortese

Claudio Giberti, Fabrizio Gallo, Maurizio Schenone, Pierluigi Cortese, Department of Urology, San Paolo Hospital, 17100 Savona, Italy

Author contributions: Giberti C, Gallo F and Schenone M planned and performed the surgical procedure; Cortese P provided the figures; Gallo F wrote the manuscript; all the authors were involved in editing the manuscript and had read, revised and approved the final draft.

Correspondence to: Fabrizio Gallo MD, Department of Urology, San Paolo Hospital, Via Genova, 30, 17100 Savona, Italy. fabrizio.gallo@fastwebmail.it

Telephone: +39-34-79043036 Fax: +39-19-8404447

Received: December 31, 2013 Revised: February 14, 2014
Accepted: April 17, 2014
Published online: June 16, 2014

Abstract

We report our first simultaneous bilateral robot assisted partial nephrectomy (RAPN) in order to show and critically discuss the feasibility of this procedure. Materials and methods A 69-year-old male patient visited our department due to incidental finding of bilateral mesorenal small masses (2.5 cm on the right and 3.5 cm on the left) suspicious for malignancy. We started from the right side with patient in flank position. Port placement: 12-mm periumbilical camera port, two 8-mm robotic ports in wide "V"configuration, additional 12 mm assistant port on the midline between the umbilicus and symphysis pubis. A right unclamping RAPN with sliding clip renorrhaphy was performed. The trocars were removed and the robot undocked. Without interrupting the anesthesiological procedures, the patient was reported in supine position and, after 180 degrees rotation of the surgical bed, was newly placed in contralateral flank position. Using both the previous periumbilical and midline ports, two other 8-mm robotic trocars were placed. The robot was then redocked and RAPN was also performed on the left side using the same previously reported technique. Results Total time: 285 min. Estimated blood losses: 150 cc. Postoperative period: uneventful. Pathological examination: bilateral renal cell carcinoma, negative surgical margins. Conclusions Our experience was encouraging and confirmed the feasibility and safety of this procedure. The planning of our technique was time and cost effective with cosmetic benefit for the patient. However, we think that an appropriate selection of the patients and a skill in robotic renal surgery are advisable before approaching this type of surgery.

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Key words: Robotics; Nephrectomy; Renal cell carcinoma; Remote operation robotics

Core tip: Very few papers have been reported concerning simultaneous bilateral robot assisted partial nephrectomy. We think that our technique was noteworthy for some important aspects: the number of the ports was minimized, the disposition of the operatory room allows the quick rotation of the patient's bed and the redocking of the robot, the operative time was acceptable, the unclamping technique decreased the risk of renal insufficiency, the cost for two nephrectomies was decreased. In conclusion, our technique was safe, feasible, time and cost effective with a cosmetic benefit for the patient.

Giberti C, Gallo F, Schenone M, Cortese P. Simultaneous bilateral robotic partial nephrectomy: Case report and critical evaluation of the technique. *World J Clin Cases* 2014; 2(6): 224-227 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i6/224.htm DOI: http://dx.doi.org/10.12998/wjcc.v2.i6.224

INTRODUCTION

In the last few years, robot-assisted partial nephrectomy (RAPN) has become a promising procedure able to

bridge the technical difficulties of laparoscopic partial nephrectomy (LPN), permitting a broader diffusion of laparoscopic treatment of renal masses[1-3].

In fact, the 3D vision, the optical magnification and the robotic instruments allow surgeons to realize very precise tumor resection and to simplify the reconstructive steps of the procedure, minimizing the potential risks due to the ischemia time.

More recently, the expanded role of robot-assisted surgery has also included the simultaneous treatment of bilateral renal tumors[4,5].

This type of procedure, which is certainly fascinating, still needs to be well defined regarding the indications and the technique. We report our first case of simultaneous bilateral robotic partial nephrectomy in order to show the feasibility of our technique and critically discuss both the advantages and the disadvantages of this procedure.

CASE REPORT

Patient

A 69-year-old male patient visited our department due to the incidental finding of bilateral small renal masses. Magnetic resonance scans showed a 2.5 cm mass in the middle portion of the right kidney and a 3.5 cm mass in the middle portion of the left kidney with no involvement of the collecting systems. The two masses were suspicious for malignancy (Figure 1). The differential diagnosis was made with benign tumors and complicated cysts. There was no past surgical history. General physical examination and the preoperative exams were normal. The body mass index (BMI) was 23.51.

Surgical technique

The operating theatre was set up as shown in Figure 2. The procedure was performed using a three-arm Da Vinci Robot, standard version, starting from the right side. The patient was secured in a flank position with the table slightly bent. Regarding the port placement, a 12-mm periumbilical port was placed for the camera. Two 8-mm robotic instrument ports were placed approximately 8 cm from the camera in a wide "V" configuration centered on the renal tumor. An additional 12 mm assistant trocar was placed on the midline between the umbilicus and symphysis pubis (Figure 3). A 30° angle lens was used. The robotic instruments included bipolar fenestrated forceps, monopolar cautery scissors, and two needle drivers. The peritoneum was incised sharply along the line of Toldt and the bowel was mobilized medially exposing the Gerota's fascia. The renal artery and vein were isolated and vessel loops were placed around them. The Gerota's fascia was dissected off the surface of the kidney and the kidney was extensively mobilized until easy access to the tumor was achieved from all sides. The RAPN was then performed without hilar clamping. The renal specimen was retrieved using an endobag. The inner defect was closed with a running outside-in Monocryl 4-0 suture preloaded with a Hem-o-lok clip, taking care to include retracted vessels or calyces into the suture. The borders

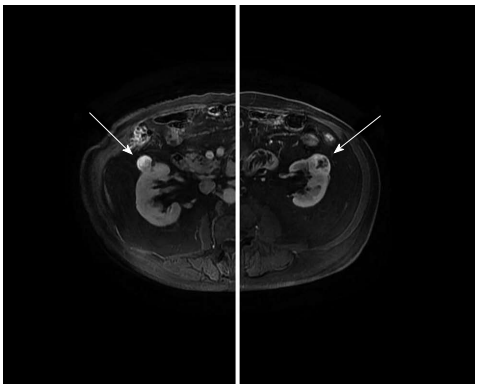

Figure 1 Magnetic resonance scans. The right and left small renal masses are indicated.

of the defect were closed with another running outside to inside Monocryl 2-0 suture including a haemostatic agent and secured with Hem-o-lok clips at each bite. Through the sliding clip technique, the right tension was brought on these sutures[6].

The Gerota's fascia and the peritoneum were closed. A wound drain was introduced through the inferior 8-mm port. All the trocars were removed and the robot was undocked.

Without interrupting the anesthesia, the patient was repositioned in the supine position and, after a 180 degree rotation of the surgical bed, he was placed in the contralateral flank position. Using both the previous periumbilical and midline ports for the camera and the additional 12-mm assistant trocar, respectively, the other 8-mm robotic trocars were placed in a wide "V"configuration centered on the left renal tumor (Figure 3). The robot was then redocked without changing any disposition of the instruments, the furniture or the staff inside the operating theatre.

A second RAPN without hilar clamping was also performed on the left side following the previously reported technique. Intraoperative ultrasonography was used on this side in order to score the margins of the lesion.

The total operation time was 285 min and total console time was 240 min. The estimated blood loss was 150 cc. The postoperative period was uneventful. The patient was mobilized on day 2. The urethral catheter was removed on day 2. The right and left drains were removed on days 2 and 3, respectively. The patient was discharged on day 4. The pathological examination reported bilateral renal cell carcinoma, Fuhrman grade 1, with negative surgical margins. Six months after surgery, computed tomography scan did not show tumor local recurrences.

DISCUSSION

The robotic approach for conservative renal surgery is becoming increasingly common due to the reported encouraging outcomes in terms of safety, feasibility and efficacy of this procedure[1-3,7]. Very few papers have been reported in literature concerning simultaneous bilateral RAPNs probably due to the low incidence of bilateral re-

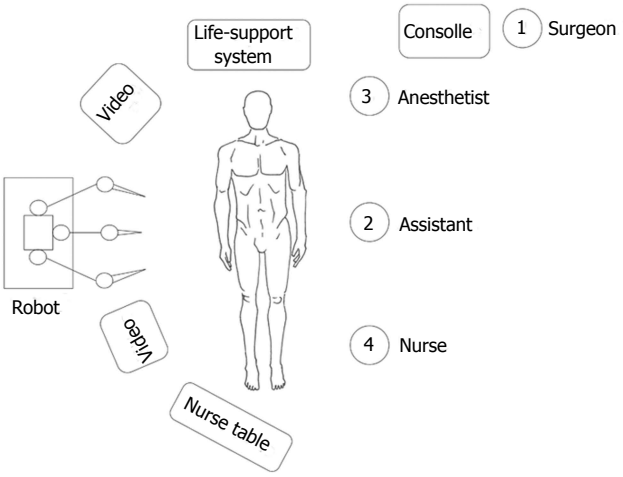

Figure 2 Disposition of the operating theatre.

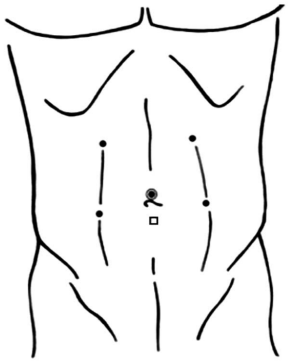

Figure 3 Port placements. Four 8-mm robotic ports (black circles), one 12-mm periumbilical camera port (double circle) and one 12-mm midline port (square).

nal tumors but also due to the difficulties of this type of surgery[4-5]. The potential benefits of simultaneous bilateral surgery could be related to the advantages of a unique surgical procedure with single anesthesia, shorter overall hospitalization, faster overall recovery and lower costs than two separate procedures. Furthermore, a cosmetic benefit due to the reuse of some ports for both sides could be considered. However, these advantages could be balanced by the risks of longer total anesthesia time, higher blood loss and postoperative renal insufficiency. In this setting, the procedure should be planned appropriately in order to maximize the benefits and minimize the risks. We think that our technique was noteworthy for some aspects.

The positioning of the ports was planned accurately in order to minimize the number of the abdominal incisions. In particular, the camera and the assistant ports were positioned on the xifopubic line and used for both sides. In the end, the bilateral RAPN was performed using only six ports.

The disposition of the operating theatre was studied in order to allow the rotation of the patient's bed without changing the positions of the robot, the instruments and the operators. This detail allowed us to undock and redock the robot very quickly between the two nephrectomies, avoiding the waste of precious minutes.

Overall, the entire operation lasted less than 5 h including anesthesiological procedures, patient positioning and trocar placement. We think that this is an acceptable anesthesia time for a bilateral procedure as confirmed by the regular observations made in the postoperative period.

The surgical technique with no arterial clamping decreased the risk of postoperative renal insufficiency which can be more frequent after a bilateral procedure, especially when bilateral clamping is performed, as already reported in literature[4].

This procedure was really cost effective. In fact, the two nephrectomies were performed without shutting the robot down and using the same surgical instruments. These aspects helped strongly to decrease the costs of a robotic operation.

Some limitations of our technique should be mentioned. In fact, an appropriate selection of the patients (mainly regarding the size, the location of the tumors or the preexisting condition of chronic renal insufficiency) and a very good skill in renal robotic surgery are really advisable before approaching this type of surgery.

In conclusion, our experience was encouraging and confirmed the feasibility and the safety of this procedure. Furthermore, the planning of our technique was time and cost effective with a cosmetic benefit for the patient. However, we think that an appropriate selection of the patients and skill in robotic renal surgery are really advisable before approaching this type of surgery.

ACKNOWLEDGMENTS

We thank Dr. Jennifer McDermott for the language revision.

COMMENTS

Case characteristics

A 69-year-old male patient visited our department due to the incidental finding of bilateral small renal masses.

Clinical diagnosis

The general physical examination was normal, the renal masses were not palpable. The body mass index was 23.51.

Differential diagnosis

Benign tumors, complicated cysts.

Laboratory diagnosis

The preoperative exams were normal.

Imaging diagnosis

Magnetic resonance scans showed a 2.5 cm mass in the middle portion of the right kidney and a 3.5 cm mass in the middle portion of the left kidney with no involvement of the collecting systems.

Pathological diagnosis

The pathological examination reported bilateral renal cell carcinoma, Fuhrman grade 1, with negative surgical margins.

Treatment

A simultaneous bilateral robotic partial nephrectomy was performed.

Related reports

The surgical treatment of small renal masses is well known using different approaches (mainly open surgery, laparoscopy and cryotherapy). However, very few papers have been reported in literature concerning simultaneous bilateral

robot assisted partial nephrectomy probably due to the low incidence of bilateral renal tumors but also due to the difficulties of this type of surgery.

Experiences and lessons

This case report showed the feasibility, the safety, the time and cost effectiveness of this procedure with a cosmetic benefit for the patient.

Peer review

This is a well written and interesting case.

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P- Reviewers: Gbolade BA, Sanju G **S- Editor**: Qi Y
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DOI: 10.12998/wjcc.v2.i6.228

World J Clin Cases 2014 June 16; 2(6): 228-231
ISSN 2307-8960 (online)
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CASE REPORT

Cabazitaxel in castration resistant prostate cancer with brain metastases: 3 case reports

Sabino De Placido, Pasquale Rescigno, Piera Federico, Carlo Buonerba, Davide Bosso, Livio Puglia, Michela Izzo, Tania Policastro, Giuseppe Di Lorenzo

Pasquale Rescigno, Piera Federico, Carlo Buonerba, Davide Bosso, Livio Puglia, Michela Izzo, Tania Policastro, Sabino De Placido, Giuseppe Di Lorenzo, Genitourinary Cancer Section, Medical Oncology, Department of Clinical Medicine, Federico II University, Napoli 80140, Italy
Author contributions: De Placido S and Rescigno P contributed to the drafting of the manuscript; Federico P, Buonerba C, Bosso D, Puglia L, Izzo M and Policastro T contributed to the acquisition of the data; De Placido S and Di Lorenzo G contributed to the critical revision of the manuscript.
Correspondence to: Giuseppe Di Lorenzo, MD, PhD, Genitourinary Cancer Section, Medical Oncology, Department of Clinical Medicine, Federico II University, Corso Umberto Ⅰ, Napoli 80140, Italy. giuseppedilorenzoncol@hotmail.com
Telephone: +39-081-7463660 Fax: +39-081-2203147
Received: January 5, 2014 Revised: March 31, 2014
Accepted: May 8, 2014
Published online: June 16, 2014

Abstract

Prostate cancer is the most common non-cutaneous malignancy for men. The skeleton is the most common metastatic site but, following an improvement in survival, metastases in uncommon sites are being found more frequently in clinical practice, especially brain metastases. Despite the new drugs now available for metastatic castration resistant prostate cancer, no clinical evidence exists about their effectiveness on brain metastases. We describe the clinical history of 3 patients treated with cabazitaxel plus whole brain radiotherapy. These case reports demonstrate that cabazitaxel is highly active and well tolerated in brain metastases.

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Key words: Cabazitaxel; Brain metastases; Prostate cancer

Core tip: Due to the improvement in terms of survival, the incidence of brain metastases (BMs) has increased in patients with metastatic castration resistant prostatic cancer (mCRPC). Despite the large number of treatments now available, the prognosis of patients with BMs is still poor. First, we demonstrate the efficacy of cabazitaxel on brain mestastases in three CRPC patients and then show its profile of tolerability in combination with whole brain radiotherapy.

De Placido S, Rescigno P, Federico P, Buonerba C, Bosso D, Puglia L, Izzo M, Policastro T, Di Lorenzo G. Cabazitaxel in castration resistant prostate cancer with brain metastases: 3 case reports. *World J Clin Cases* 2014; 2(6): 228-231 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i6/228.htm DOI: http://dx.doi.org/10.12998/wjcc.v2.i6.228

INTRODUCTION

Prostate cancer (PC) is the most common non-cutaneous malignancy for men, with an estimated number of new cases of 241740 in 2013 in the United States[1]. Nevertheless, PC is not the leading cause of death in the male population due to its ability to rarely metastasize to organs other than bones[2].

Although the skeleton remains the most common metastatic site, the availability of new active drugs for metastatic castration resistant prostatic cancer (mCRPC) has changed the natural history of this disease, leading to a considerable improvement in survival so that metastases in previously considered uncommon sites are now found more frequently[3].

Brain is the site of metastases in almost 12% of cases with a poor prognosis at their appearance[4].

Despite an increased incidence of BMs, the impact of new drugs for mCRPC on this metastatic site remains poorly understood. First of all, patients with BMs are not routinely enrolled in phase Ⅲ clinical trials and there are

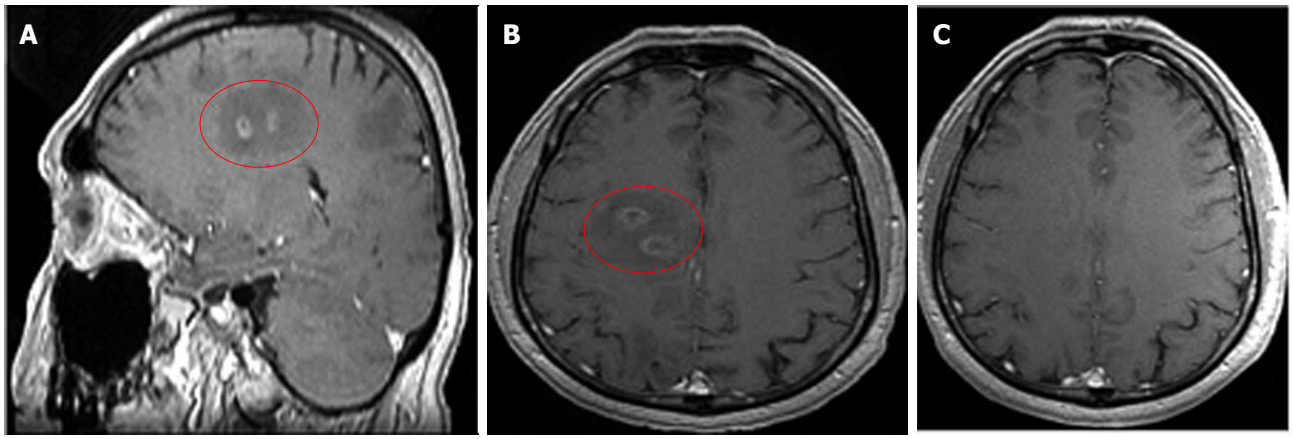

Figure 1 Show a complete response in the brain lesions before and after 6 cycles of cabazitaxel. A, B: Brain metastases before cabazitaxel; C: Complete response after 6 cycles of cabazitaxel.

no prospective and ad-hoc studies in this particular setting. Actually, there is only preclinical data showing that cabazitaxel is able to pass the brain-blood barrier (BBB)[5] but no evidence about its efficacy in humans.

Otherwise, there is also little data concerning the role of radiation therapy on the treatment of BMs from PC which seems to have only a palliative intent[6]. Here, we describe three case reports of brain metastases in CRPC patients who were treated with cabazitaxel plus whole brain radiotherapy.

CASE REPORT

The patients were 70, 70 and 72 years old. All patients presented at diagnosis with a high risk disease (Table 1). Patients 1 and 2 did not receive primary treatment because bone metastases and lymph node metastases were detected with bone and computed tomography scans. These 2 patients began hormonal therapy with luteinizing hormone releasing hormone analogue (aLHRH) first and then with complete androgen blockage (CAB), adding bicalutamide 50 mg.

Patient 3 underwent prostatectomy and radiotherapy for locally advanced disease. The disease progressed after 5 mo due to the appearance of bone metastases and aLHRH was started. All patients had a long androgen deprivation therapy (ADT) history (36-50 mo). Docetaxel was first line chemotherapy with a progression free survival (PFS) of 7, 7 and 11 mo respectively (Table 1). Patient 3 was treated with abiraterone as second line treatment and progressed after 6 mo.

The patients presented with multiple BMs (in number, 2, 3 and 3 respectively) confirmed with a magnetic resonance imagining (MRI) before starting cabazitaxel and the liver and lung were the other metastatic sites (Table 1). A total of 30 cycles of cabazitaxel were administered at standard dose without reductions (Table 1). Contemporaneous whole brain radiotherapy was performed at the dose of 30 Gy.

Patient 3 obtained a complete response on brain and liver metastases with a PSA reduction of 90% after 6 cycles (Figure 1), while two partial responses in brain (the lesions were halved) and lung were observed, with a PSA decrease of 40% after 6 cycles for patient 1 and 2.

No grade 3-4 toxicities were experienced; all patients received pegylated granulocyte colony stimulating factor (PEG-G-CSF) to prevent febrile neutropenia. The most important non-hematological toxicities were grade 2 nausea and asthenia.

The PFS of patients 1 and 2 was 7 and 13 mo while patient 3 is still progression-free. Patients 1 and 2 received further therapy after cabazitaxel (abiraterone and platinum regimen) and died after 3 mo.

DISCUSSION

BM appearance is a rare and terminal event in the natural history of PC due to greater aggressiveness and poor response to common therapies. BMs are often essentially single, supratentorial and occur with nonfocal neurological symptoms related to intracranial hypertension. A retrospective study of 103 patients with BMs showed that radiotherapy alone is an effective treatment with a median survival of 3.5 mo[7].

Further improvement in survival was noted in five patients who underwent stereotactic radiosurgery (SRS). Although no complete responses were obtained, symptoms improved[8].

BMs are more frequent in the CRPC setting than in the past due to the availability of new drugs and longer survival of metastatic patients. In the docetaxel era, the prognosis of patients with BMs was still poor and median survival was only 8 weeks after BM diagnosis, demonstrating the clinical ineffectiveness of docetaxel[3].

Among the new approved drugs for mCRPC, such as cabazitaxel, abiraterone, enzalutamide and sipuleucel-T, only cabazitaxel has been shown to be able to pass the BBB. Cisternino and colleagues observed a non-linear accumulation of cabazitaxel in the brains of rats, occurring by saturation of the P-glycoprotein in the BBB[5].

Table 1 Patient characteristics

	Patient 1	Patient 2	Patient 3
Age (yr)	70	70	72
Comorbidities	Hypertension	Hypertension	Diabetes
Primary treatment	Hormonal therapy	Hormonal therapy	Surgery and radiation therapy
Gleason score	8 (4 + 4)	8 (4 + 4)	8 (5 + 3)
PSA at baseline[1] (ng/mL)	158	82	17
ADT time (mo)	38	36	50
Docetaxel cycles	12	8	8
PSA pre-cabazitaxel (ng/mL)	95	292	140
Sites of metastases	Bone, lung, brain	Bone, lung, brain	Bone, liver, brain
Cabazitaxel cycles	12	8	10
Best response	PR on brain and lung	PR on brain and lung	CR on liver and brain
Toxicities	Anemia grade 1, asthenia grade 2	Nausea grade 2; neutropenia grade 2	Asthenia grade 2

[1]Before primary treatment. ADT: androgen deprivation therapy; PR: Partial response; CR: Complete response.

These 3 case reports describe the role of cabazitaxel in patients with BMs for the first time and the results are encouraging for 3 reasons.

Firstly, it shows the definite efficacy of cabazitaxel in BMs with an amazing PFS compared with the Tropic trial PFS[9]. Secondly, the association of whole brain radiotherapy and chemotherapy with cabazitaxel gives better results in terms of radiological response and survival than the data presented above.

Thirdly, the combination does not seem to be particularly toxic, especially in terms of hematological toxicities. We administered preventive PEG-G-CSF and, as previously shown in an Italian study, it reduced the grade 3 and 4 neutropenia reported with cabazitaxel[10]. Of note, all three patients had a gleason 8 at diagnosis, which is consistent with our previosly reported findings suggesting improved PFS in patients with high gleason score receiving cabazitaxel[11].

Our case reports demonstrate that cabazitaxel improved PFS and overall survival in our patients with BMs and is well tolerated in combination with we decided to report these cases in a full paper without presenting them as a meeting abstract, considering that only 50% of abstracts are subsequently published as full papers[12]. The lack of ad-hoc studies and the exclusion of men with brain metastases from phase Ⅲ trials make our data the first evidence in this field. Prospective trials are needed to confirm our preliminary results.

COMMENTS

Case characteristics

All patients presented at diagnosis with a high risk disease.

Treatment

The authors demonstrate the efficacy of cabazitaxel on brain metastases in three castration resistant prostatic cancer patients and show its profile of tolerability in combination with whole brain radiotherapy.

Experiences and lessons

These case reports demonstrate that cabazitaxel is highly active and well tolerated in brain metastases.

Peer review

Nice, well written paper with interesting data potentially useful in the clinical setting.

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World J Clin Cases 2014 June 16; 2(6): 232-234
ISSN 2307-8960 (online)
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CASE REPORT

Gastrointestinal perforation due to incarcerated Meckel's diverticulum in right femoral canal

Yusuf Yagmur, Sami Akbulut, Mehmet Ali Can

Yusuf Yagmur, Sami Akbulut, Mehmet Ali Can, Department of Surgery, Diyarbakir Education and Research Hospital, 21400 Diyarbakir, Turkey
Author contributions: Yagmur Y and Akbulut S designed the report; Akbulut S and Can MA performed surgical operation; Akbulut S and Yagmur Y organized the report and wrote paper.
Correspondence to: Sami Akbulut, MD, FICS, FACS, Department of Surgery, Diyarbakir Education and Research Hospital, 21400 Diyarbakir, Turkey. akbulutsami@gmail.com
Telephone: +90-412-2580075 Fax: +90-412-2580070
Received: December 26, 2013 Revised: February 11, 2014
Accepted: April 9, 2014
Published online: June 16, 2014

Abstract

Meckel's diverticulum is a very common congenital anomaly of the gastrointestinal tract but many cases remain asymptomatic and are diagnosed incidentally during laparoscopic or other surgical procedures. Cases of femoral hernia involving Meckel's diverticulum are rare, with less than 50 cases reported in the literature since Littre published the first description of this coincident condition over 300 years ago. While all true "Littre's hernias" contain a Meckel's diverticulum, the involved anatomical sites are various, the most common being the inner groin (inguinal), the outer groin (femoral), and the belly button (umbilical). Complications of Littre's hernias include incarceration, strangulation, necrosis, and perforation. Herein, we describe a case of Littre's hernia that involved an incarcerated Meckel's diverticulum in a femoral hernia that was diagnosed upon investigation of symptomology manifesting from perforation and was successfully managed by surgical resection with stapler devices.

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Key words: Meckel's diverticulum; Incarceration; Littre Hernia; Gastrointestinal perforation

Core tip: Meckel's diverticulum is most commonly diagnosed congenital anomaly of the gastrointestinal tract. Any hernia containing a Meckel's diverticulum is designated as a Littre's hernia. Although rare in overall incidence, the most common complications of Littre's hernias are perforation, bowel obstruction secondary to strangulation, and incarceration within the hernial sac. In this case study, we present and share the diagnosis and successfully management of a case of incarcerated Meckel's diverticulum in a femoral hernia (Littre's hernia) with perforation.

Yagmur Y, Akbulut S, Can MA. Gastrointestinal perforation due to incarcerated Meckel's diverticulum in right femoral canal. *World J Clin Cases* 2014; 2(6): 232-234 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i6/232.htm DOI: http://dx.doi.org/10.12998/wjcc.v2.i6.232

INTRODUCTION

The most commonly diagnosed congenital abnormality of the small intestine, Meckel's diverticulum, occurs when a portion of the vitelline duct fails to properly regress into the antimesenteric border of the terminal ileum at the end of the seventh week of gestation. As such, a Meckel's diverticulum contains all layers of the intestinal wall and is classified as a true diverticulum[1-6]. Estimates of the incidence of this condition have ranged from 0.3% to 3%, but its frequent asymptomatic nature may belie its true rates among the general population. Meckel's diverticula can be diagnosed as incident findings in laparoscopic or laparotomic examinations for unexplained symptoms or other conditions that have manifested clinical symptoms. Any hernia containing a Meckel's diverticulum is designated as a Littre's hernia. The most common sites of these coincident anatomical abnormalities are the inner groin (inguinal hernia, approximately 50%), the outer groin (femoral hernia, approximately 20%), and the belly button (umbilical hernia, approximately 20%).

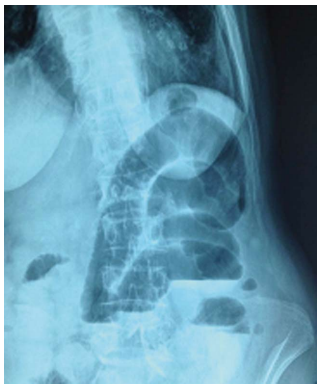

Figure 1 Air-fluid levels detected by abdominal radiography. The findings were compatible with intestinal obstruction.

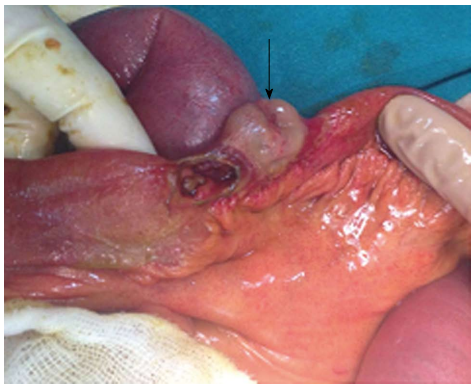

Figure 2 Perforation area proximal to the Meckel's diverticulum. The Meckel's diverticulum is indicated by a black arrow.

Although rare in overall incidence, the most common complications of Littre's hernias are perforation, bowel obstruction secondary to strangulation, and incarceration within the hernial sac[1]. Herein, we report the diagnosis and successfully management of a case of incarcerated Meckel's diverticulum in a femoral hernia (Littre's hernia) with ileal perforation.

CASE REPORT

A 73-year-old female presented to our Emergency Department with severe abdominal pain, nausea, and vomiting; the symptoms had begun one week previous, but had increased in severity over the last two days. The patient also reported the last instances of flatulence and defecation being three days previous. Results of standard laboratory blood tests were normal, hemoglobin: 13.2 g/dL (normal range, NR: 12.5-16.0), blood urea nitrogen: 22 mg/dL (NR: 10-50 mg/dL), and creatinine: 0.5 mg/dL (NR: 0.4-1.2 mg/dL), with the exception of a high white blood cell count [16000/μL (NR: 4100-11200/μL)] and neutrophil ratio [89% (NR: 50%-78%)]. In physical examination, auscultation revealed obstructive and hyperactive bowel sounds and palpation revealed serious tenderness with rebound pain that was particularly robust in the right lower quadrant. Abdominal radiographic examination showed air-fluid levels (Figure 1). Nasogastric decompression was performed in the Emergency Department. The collected clinical symptoms and findings from biochemical analysis and radiological examination suggested acute mechanical small bowel obstruction possibly related to internal herniation or perforation. The patient was prepared for exploration. Laparotomy with a midline incision revealed a Meckel's diverticulum with an ileal segment incarcerated in the right femoral canal and which had perforated the antimesenteric border of the ileum (Figure 2). The abnormality was managed by first removing the diverticulum through the femoral canal, suturing the femoral canal closed, resecting the partial ileal segment that included the perforated ileal segment and Meckel's diverticulum (15 cm in length), and creating a side-to-side anastomosis with stapler devices (Endo-GIA Stapling System; Covidian, Dublin, Ireland). Postoperative recovery was uncomplicated and the patient was discharged to home. Pathological analysis of the resected tissues showed no signs of gastric or pancreatic metaplasia.

DISCUSSION

In the first five weeks of normal gestational development, the Meckel's diverticulum is the intestinal portion of the omphaloenteric duct through which the midgut communicates with the umbilical vesicle. Located at the antimesenteric border of the ileum, about 30 to 90 cm from the ileocecal valve, it can measure between 3 and 6 cm in length and is usually approximately 2 cm in diameter[2]. Failure of the tissue to regress by gestational week 7 is not fatal, and the congenital abnormality may remain asymptomatic (and undetected) throughout life. The lifetime complication rate estimated to be approximately 4%, with a higher incidence in males[4], and the most common complications are bleeding, inflammation, and obstruction[5].

Cases of femoral hernia involving Meckel's diverticulum are rare, with less than 50 cases reported in the literature since Littre published the first description of this coincident condition over 300 years ago[2]. A true Littre's hernia contains a Meckel's diverticulum alone, but cases of mixed Littre's hernia containing ileum or other abdominal viscera have been reported[3]. Perforation of the Meckel's diverticulum may occur due to peptic ulceration or compromised circulation and luminal patency at the narrow neck of the hernia[3].

Preoperative diagnosis of a strangulated Littre's hernia is unlikely, as the presenting signs and symptoms are subtler and evolve more slowly than those of strangulated small intestine. Fever, pain, and signs of intestinal obstruction occur late or not at all. Moreover, there may be no specific sign of bowel involvement, other than local inflammation surrounding the hernia until an enterocutaneous fistula develops[5]. The *septuagenarian* case described herein presented with signs of obstruction (*i.e.*, serious abdominal tenderness, rebound pain, and air-fluid levels).

In Littre's hernia, obstruction can occur if the base of the diverticulum is broad enough to cause narrowing of the intestinal lumen. Presenting symptoms are tender

mass proximal to a hernial orifice with nausea, vomiting, abdominal pain, local groin pain and swelling. Pyrexia is normally associated with strangulation[5]. Repair of the Littre's hernia consists of local diverticulum resection and herniorrhaphy. In cases of perforation, care must be taken to not contaminate the hernia field[2], and resection of the diverticulum ileal loop with end-to-end or side-to-side anastomosis is the recommended treatment.

COMMENTS

Case characteristics

A 73-year-old female presented to our Emergency Department with severe abdominal pain, nausea, and vomiting; the symptoms had begun one week previous, but had increased in severity over the last two days.

Clinical diagnosis

Auscultation revealed obstructive and hyperactive bowel sounds and palpation revealed serious tenderness with rebound pain that was particularly robust in the right lower quadrant.

Imaging diagnosis

Abdominal radiographic examination showed air-fluid levels. Laparotomy with a midline incision revealed a Meckel's diverticulum with an ileal segment incarcerated in the right femoral canal and which had perforated the antimesenteric border of the ileum

Pathological diagnosis

Pathological analysis of the resected tissues showed no signs of gastric or pancreatic metaplasia.

Treatment

Care must be taken to not contaminate the hernia field, and resection of the diverticulum ileal loop with end-to-end or side-to-side anastomosis is the recommended treatment.

Peer review

The authors described the case of gastrointestinal perforation due to incarcerated Meckel's diverticulum in right femoral canal. The authors also demonstrated the figure of air-fluid levels detected by abdominal radiography.

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P- Reviewers: Contini S, Lin JK, Masaki T, Pellicano R, Sadik R
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DOI: 10.12998/wjcc.v2.i6.235

World J Clin Cases 2014 June 16; 2(6): 235-239
ISSN 2307-8960 (online)
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CLINICOPATHOLOGICAL CONFERENCE

Facial nerve palsy, headache, peripheral neuropathy and Kaposi's sarcoma in an elderly man

Dimitrios Daoussis, Elisabeth Chroni, Athanassios C Tsamandas, Andrew P Andonopoulos

Dimitrios Daoussis, Andrew P Andonopoulos, Division of Rheumatology, Department of Internal Medicine, University of Patras School of Medicine, 26504 Patras, Greece
Elisabeth Chroni, Department of Neurology, University of Patras School of Medicine, 26504 Patras, Greece
Athanassios C Tsamandas, Department of Pathology, University of Patras School of Medicine, 26504 Patras, Greece
Author contributions: Daoussis D and Andonopoulos AP conceived the idea of the study, performed the clinical assessments and drafted the manuscript; Chroni E performed the nerve conduction studies and assisted in manuscript drafting; Tsamandas AC performed all histological assessments and assisted in manuscript drafting.
Correspondence to: Dimitrios Daoussis, MD, Assistant Professor of Internal Medicine/Rheumatology, Division of Rheumatology, Department of Internal Medicine, University of Patras School of Medicine, Rion, 26504 Patras, Greece. jimdaoussis@hotmail.com
Telephone: +30-2613-603693 Fax: +30-2610-993982
Received: February 12, 2014 Revised: March 26, 2014
Accepted: April 17, 2014
Published online: June 16, 2014

Abstract

We present a case of an elderly man, who initially presented with right facial nerve palsy, ipsilateral headache, elevated erythrocyte sedimentation rate (ESR) and no fever. A presumptive diagnosis of giant cell arteritis was made and the patient was treated with high-dose steroids. A temporal artery biopsy was negative. Several months later, while on 16 mg of methylprednisolone daily, he presented with severe sensorimotor peripheral symmetric neuropathy, muscle wasting and inability to walk, uncontrolled blood sugar and psychosis. A work-up for malignancy was initiated with the suspicion of a paraneoplastic process. At the same time a biopsy of the macular skin lesions that had appeared on the skin of the left elbow and right knee almost simultaneously was inconclusive, whereas a repeat biopsy from the same area of the lesions that had become nodular, a month later, was indicative of Kaposi's sarcoma. Finally, a third biopsy of a similar lesion, after spreading of the skin process, confirmed the diagnosis of Kaposi's sarcoma. He was treated with interferon α and later was seen in very satisfactory condition, with no clinical evidence of neuropathy, normal muscle strength, no headache, normal electrophysiologic nerve studies, involution of Kaposi's lesions and a normal ESR.

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Key words: Facial nerve palsy; Peripheral neuropathy; Vasculitis; Paraneoplastic syndrome; Kaposi's sarcoma

Core tip: We present a case of an elderly man, who initially presented with right facial nerve palsy, ipsilateral headache, elevated erythrocyte sedimentation rate and no fever. A presumptive diagnosis of giant cell arteritis was made and the patient was started on high-dose steroids. Several months later, he presented with severe sensorimotor peripheral symmetric neuropathy. A biopsy of the macular skin lesions that had appeared almost simultaneously, was suggestive of Kaposi's sarcoma. Although peripheral and cranial nerve involvement has not been reported in Kaposi's sarcoma, we postulate that the patient's condition could be attributed to that, within the context of a paraneoplastic process.

Daoussis D, Chroni E, Tsamandas AC, Andonopoulos AP. Facial nerve palsy, headache, peripheral neuropathy and Kaposi's sarcoma in an elderly man. *World J Clin Cases* 2014; 2(6): 235-239 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i6/235.htm DOI: http://dx.doi.org/10.12998/wjcc.v2.i6.235

INTRODUCTION

We present herein an interesting case of an elderly patient with right facial nerve palsy, ipsilateral headache, elevated erythrocyte sedimentation rate (ESR), sensorimotor neu-

ropathy and Kaposi's sarcoma.

CASE REPORT

History report

Dimitrios Daoussis, MD: The patient was a 72-year-old white man, who was initially admitted on September 16, 2009 to the department of neurology, with chief complaint of severe persistent right sided headache. Twenty days prior to admission, he experienced acutely right facial nerve palsy of peripheral type. Ten days later he developed severe right temporal headache, and was started on prednisolone 25 mg/d by oral administration (*po*) advised by a neurologist. He had noticed gradual impairment of hearing acuity since four months ago. Glaucoma had been present for the last three years, and right eye blindness for ten years, due to an accident. There was no fever, and except for right peripheral facial nerve palsy, physical examination was unremarkable. Laboratory examination revealed a hematocrit of 35.1% with mean corpuscular volume 93.4, a white blood cell (WBC) count of 12700/μL, a platelet count of 372 000/μL and a markedly elevated ESR of 105 mm/h. All biochemical parameters and his serologic profile, including rheumatoid factor, antinuclear antibodies, complement (C3 and C4) and antineutrophil cytoplasmic antibodies (ANCA) were within the normal range. Computed tomography (CT) and magnetic resonance imaging (MRI) brain scans were normal. Otolaryngologic evaluation suggested bilateral adhesive otitis, whereas ophthalmologic examination confirmed the presence of glaucoma and the right side blindness. The patient was started on methylprednisolone 48 mg/d *po*, with the presumptive diagnosis of giant cell arteritis (GCA). He was discharged on this regimen on September 24, 2009, pending the result of a right temporal artery biopsy, performed on the day of discharge.

He was first seen at the rheumatology outpatient clinic three months later, on December 20, 2009, again complaining of right temporal headache, despite of having been taking methylprednisolone 40 mg/d. The report of the temporal artery biopsy was negative for GCA and steroid tapering was started.

On February 23, 2010, he reported deterioration of his headaches, whereas his ESR was 58 mm/h. Methylprednisolone dose was increased back to 32 mg/d, and on March 22, 2010, his ESR had dropped to 6 mm/h and headaches had improved. Gradual tapering of steroids was again undertaken and an appointment after two months was given.

The patient was admitted to the department of medicine on June 26, 2010 with fatigue, profound muscle weakness, inability to walk and psychosis. While on methylprednisolone 16 mg/d, his admission glucose was 665 mg/dL, whereas it had been always normal before. He was afebrile without headache. He was profoundly wasted, with severe proximal muscle weakness and decreased deep tendon reflexes. The right facial nerve palsy had improved. Two bluish-red macules, about 1.5 cm in diameter, were noticed over the skin of the right knee and left elbow, respectively. Laboratory tests revealed a hematocrit of 29.8%, mild leukocytosis, normal platelet count, an ESR of 28 mm/h, potassium of 3.73 mg%, sodium of 136 mg%, calcium of 8.64 mg%, magnesium of 1.5 mg%, creatinine of 0.6 mg%, total serum protein of 5 g% with albumin 2 g%, and normal creatine phosphokinase, transaminases, alkaline phosphatase and normal urinalysis. Serologic profile for rheumatic diseases and A, B and C hepatitis was unrevealing. Serum angiotensin converting enzyme (SACE) level was normal. A chest X-ray was also normal. An electrophysiologic evaluation was compatible with mixed sensorimotor neuropathy, worse on the lower extremities, but no myositis was found, except for changes due to chronic steroid administration. Cerebrospinal fluid examination was normal. Proximal muscle biopsy did not disclose myositis, vasculitis or granuloma. A purified protein derivative skin test was negative. Thyroid function was also normal.

With the suspicion of an underlying occult malignancy, an extensive work- up towards that direction was initiated; gastroscopy, barium enema, brain, thoracic and abdominal CT scans, thyroid ultrasound and cancer indices including alpha fetoprotein, carcinoembryonic antigen (CEA), CA 19-9, prostatic specific antigen, beta horionic gonadotrophin were all normal. A biopsy of the left elbow skin lesion displayed atypical changes (see pathological description below).

With the patient's condition deteriorating, both clinically and in laboratory test results (Ht = 20.3%, ESR = 70 mm/h), with uncontrolled blood sugar levels, and cushingoid features, including psychosis and severe muscle wasting, in an attempt to spare steroids, we treated the patient with methotrexate 7.5 mg/wk. His condition improved and on July 24, 2000, one month after his admission, he was discharged on the above methotrexate dose, methylprednisolone 16 mg/d and insulin, to be followed closely.

On the August 23 appointment, the patient's general condition was relatively satisfactory, with significant improvement of his muscle strength, and a hematocrit of 36.6% and ESR 15 mm/h. However, the previously noted macules on the skin of the left elbow and right knee had become nodular and a biopsy of the lesion of the elbow was taken. This biopsy was indicative of Kaposi's sarcoma, although not typical. One month later, on September 27, the patient returned with similar bluish-red nodules all over his body, typical of Kaposi's sarcoma, and a third biopsy confirmed the diagnosis. The methotrexate was discontinued and the patient was admitted on October 5, 2000 to the dermatology service and started on interferon α (IFNα-2α) treatment. At that time, his hematocrit was 35% and ESR 25 mm/h, whereas serology for HIV was negative. An electrophysiologic nerve study showed definite improvement of the neuropathy. He was discharged on November 18, 2000, on IFNα and methylprednisolone 16 mg/d.

On January 9, 2011 at his outpatient visit, he was found in very good condition, with normal muscle strength and gait, very mild right temporal headache, no

Table 1 Findings of nerve conduction serial studies in this patient

Nerve	Parameter	1st study	2nd study	3rd study	Normal limits
Motor conduction					
Median	CV (ms)	46	60	60	⩾ 50
	CMAP (mV)	4	6	6	⩾ 5
	Min F-wave (ms)	32	28	27	⩽ 30
Peroneal	CV (ms)	35	40	43	⩾ 41
	CMAP (mV)	0.4	1	2	⩾ 2
	Min F-wave (ms)	-	-	54	⩽ 52
Sensory conduction					
Sural	CV (ms)	-	-	32	⩾ 40
	SAP (μV)	-	-	4	⩾ 8

CV: Conduction velocity; CMAP: Compound muscle action potential; SAP: Sensory action potential.

psychotic features, with normal deep tendon reflexes, and with only pale-brownish skin macules, remnants of involuted previous nodular lesions. A third electrophysiologic study showed further improvement of the neuropathy, and the patient was put to regular follow-up.

Electrophysiologic nerve studies

Elisabeth Chroni, MD, PhD: The findings were consistent with distal sensorimotor neuropathy of mixed-axonal and demylinating-type. On follow-up studies 5 and 7 mo later, a gradual improvement was observed.

Electromyography of facial muscles showed denervation potentials (fibrillations and positive waves) at rest and poor recruitment of motor units indicating axonal damage of facial nerve. All the above are depicted in Table 1.

Pathological description

Athanassios C Tsamandas, MD: The first biopsy revealed areas with proliferation of fibroblasts and capillaries, in the dermis (Figure 1A-D). The vessels of medium size displayed fibrosis of the wall, luminal narrowing and elastic lamina disruption without any fibrin deposition.

The second biopsy (Figure 1E-G) was consistent with early changes of Kaposi's sarcoma (macular-patch stage).

The third biopsy (Figure 1H-M) showed a well-defined nodule composed of vascular spaces and spindle cells that replaced the dermal collagen. Spindle cells and endothelial cells lining the vascular clefts were CD34 (+). A diagnosis of Kaposi's sarcoma was made.

Retrospectively, the slides were reviewed and the features in the first biopsy were suggestive of a very early Kaposi's sarcoma lesion.

DISCUSSION

Andrew P Andonopoulos, MD, FACP: It would be simplistic to guess that our patient had a Bell's palsy, independent of the neuropathy he later developed, and the malignancy which appeared almost concomitantly with the latter. The facial nerve palsy and the headache, along with the impressively elevated ESR, speak against the possibility of idiopathic Bell's palsy. Furthermore, the patient had no hypertension or diabetes. The psychotic episode that our patient suffered was probably associated with the iatrogenic Cushing.

Similarly, the whole picture and its evolution are against the possibility of a metabolic or toxic cause of the neurologic syndrome in our patient. Instead, it would be very suggestive of a vasculitic process, if not related to a paraneoplastic syndrome, due to the malignancy, which appeared almost simultaneously with the peripheral neuropathy.

At a first glance, there was a very strong diagnostic possibility of GCA at the time of the initial presentation of this elderly gentleman, with temporal headache and an impressively elevated ESR. The negative biopsy, despite the fact that the specimen was meticulously examined and even recut later, does not rule out this possibility, because the procedure is not 100% sensitive[1]. However, one could argue that the response of the headache to the treatment was not the one typically expected in GCA, as it was mentioned. The apparent response of the ESR to steroid administration, does not necessarily mean that the elevated ESR was secondary to GCA, because similar responses may be encountered in several vasculitic or even other different processes. Furthermore, the development of neuropathy secondary to GCA, on steroid treatment, would be unusual. Peripheral neuropathy has been described in GCA as a rather rare manifestation, coincident with clinically active disease. In a study by Caselli *et al*[2] out of 166 consecutive patients with GCA, 23 had clinical evidence of peripheral neuropathic disease, of whom 11 had a generalized peripheral neuropathy, nine had mononeuritis multiplex and three had a mononeuropathy. Only a few reports of facial nerve palsy in the context of GCA appear in the literature[3-7].

Wegener's granulomatosis is, among the vasculitides, the one that can involve most commonly cranial nerves, besides causing peripheral neuropathy[8]. Our patient had no features of Wegener's granulomatosis, without upper airway, lung or kidney involvement, and negative ANCA.

Polyarteritis nodosa would be another remote possibility, but the absence of fever, hypertension and kidney involvement, along with the negative, for vasculitis, muscle biopsy argue against this possibility[9]. Churg-Strauss syndrome could not account for our patient's picture, because the hallmarks of this entity were absent[10].

Isolated granulomatous vasculitis of the central nervous system could have been another possibility. The advanced age of our patient, the impressively high ESR, and especially the normal brain MRI and cerebrospinal fluid argue strongly against this diagnosis[11]. Finally, our patient had no evidence of sarcoidosis, with normal chest X-ray, normal SACE and negative for non-caseating granuloma muscle biopsy.

Keeping all the above considerations in mind, one then should try to correlate the patient's manifestations with the malignant disease which he was found to have.

Axonal peripheral neuropathy, such as this patient had, can be a feature of a paraneoplastic syndrome,

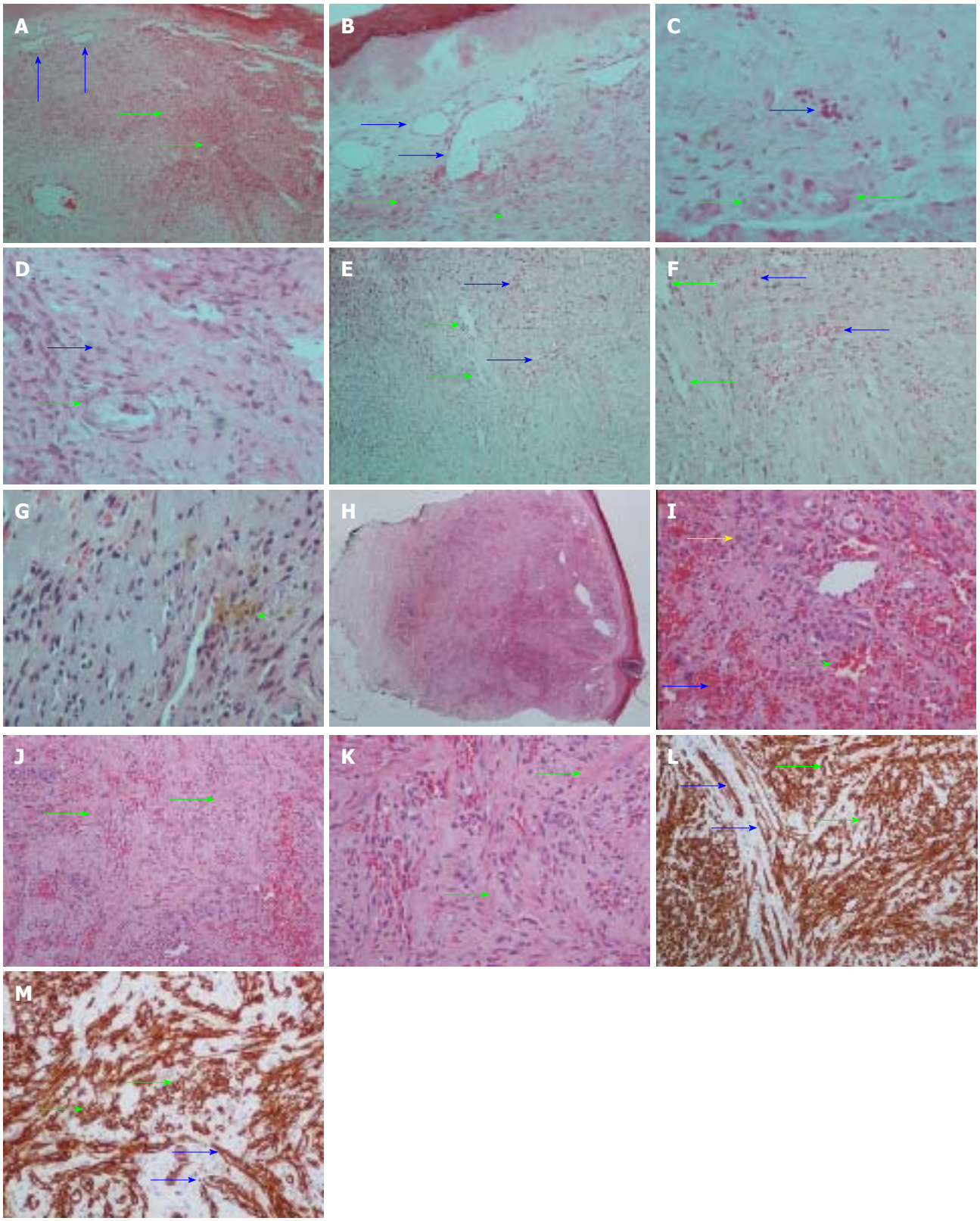

Figure 1 Microphotographs. A, B: The first skin biopsy showing proliferation of fibroblasts (green arrows) and capillaries (blue arrows) in the dermis (H and E, A, × 100; B, × 200); C: The same biopsy showing narrowing of the lumen of medium size vessels (green arrow) and blood cell extravasation (blue arrow) (H and E, × 200); D: The same biopsy showing lumen narrowing of a medium size vessel (green arrows) and fibroblast proliferation (blue arrow) (H and E, × 400); E, F: The second skin biopsy showing dilated and irregular vessels (green arrows) and blood extravasation (blue arrows). (H and E, E, × 100; F, × 200); G: The same biopsy showing the presence of siderophages (green arrow) (H and E, × 400); H: The third skin biopsy showing a well-defined nodule composed of vascular spaces and spindle cells that has replaced the dermal collagen (H and E, × 20); I: At a higher magnification, there are blood-filled vessels (green arrow), spindle cells (yellow arrow) and blood cell extravasation (blue arrow) (H and E, × 200); J, K: The same biopsy showing compartmentalization of the nodule by bands of fibrocollagen tissue (green arrows); L, M: The same biopsy showing that spindle cells (green arrows) and endothelial vascular cells (blue arrows) expressed CD34. Streptavidin-biotin perixidase (L, × 100; M, × 200).

shared by several malignancies, mainly of the lung, but of other organs as well. A meticulous search of the literature did not result in the finding of any report of Kaposi's sarcoma associated with a paraneoplastic picture from the nervous system, nor with any related to direct peripheral nerve invasion by this particular malignancy. However, this possibility cannot be excluded in our patient, and in view of our inability to find another cause responsible for his symptoms, if we wanted to put everything under one basic pathogenetic process, it would be very tempting to attribute the whole picture to the underlying malignancy.

On the other hand, it would be very difficult to accept that Kaposi's sarcoma in our patient developed iatrogenically, following immunosuppression. Kaposi's sarcoma may be caused by iatrogenic immunosuppression, but this has been mainly reported in transplant patients, receiving heavy immunosuppressive treatment with cyclosporin A and steroids with azathioprine. In addition, our patient must have Kaposi's lesions, although not diagnosed, as it could be documented from the presentation, before methotrexate was started.

In conclusion, although peripheral and cranial nerve involvement has not been reported in Kaposi's sarcoma, we postulate that the patient's condition could be attributed to this within the context of a paraneoplastic process.

COMMENTS

Case characteristics

A 72-year-old man with ipsilateral headache, skin lesions and neuropathy.

Clinical diagnosis

Facial nerve pulsy and peripheral neuropathy.

Differential diagnosis

Giant cell arteritis (GCA) or other systemic vasculitis *vs* paraneoplastic manifestations in the context of Kaposi's sarcoma.

Laboratory diagnosis

Raised inflammatory markers and anemia.

Imaging diagnosis

Negative computed tomography (CT) scan of thorax-abdomen, negative brain CT and magnetic resonance imaging.

Pathological diagnosis

Biopsies of skin lesions diagnostic of Kaposi's sarcoma.

Treatment

Initial treatment with high-dose steroids with the diagnosis of GCA. Following diagnosis of Kaposi's sarcoma, the patient was successfully treated with interferon.

Experiences and lessons

A paraneoplastic process may present with atypical manifestations.

Peer review

Although peripheral and cranial nerve involvement has not been reported in Kaposi's sarcoma, authors postulate that the patient's picture could be attributed to this within the context of a paraneoplastic process. This is a very interesting case.

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World J Clin Cases 2014 June 16; 2(6): I-V
ISSN 2307-8960 (online)
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The primary task of *WJCC* is to rapidly publish high-quality Autobiography, Case Report, Clinical Case Conference (Clinicopathological Conference), Clinical Management, Diagnostic Advances, Editorial, Field of Vision, Frontier, Medical Ethics, Original Articles, Clinical Practice, Meta-Analysis, Minireviews, Review, Therapeutics Advances, and Topic Highlight, in the fields of allergy, anesthesiology, cardiac medicine, clinical genetics, clinical neurology, critical care, dentistry, dermatology, emergency medicine, endocrinology, family medicine, gastroenterology and hepatology, geriatrics and gerontology, hematology, immunology, infectious diseases, internal medicine, obstetrics and gynecology, oncology, ophthalmology, orthopedics, otolaryngology, pathology, pediatrics, peripheral vascular disease, psychiatry, radiology, rehabilitation, respiratory medicine, rheumatology, surgery, toxicology, transplantation, and urology and nephrology.

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Name of journal

World Journal of Clinical Cases

ISSN

ISSN 2307-8960 (online)

Launch date

April 16, 2013

Frequency
Monthly

Editors-in-Chief
Giuseppe Di Lorenzo, MD, PhD, Professor, Genitourinary Cancer Section and Rare-Cancer Center, University Federico II of Napoli, Via Sergio Pansini, 5 Ed. 1, 80131, Naples, Italy

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Sandro Vento, MD, Department of Internal Medicine, University of Botswana, Private Bag 00713, Gaborone, Botswana

Shuhei Yoshida, MD, PhD, Division of Gastroenterology, Beth Israel Deaconess Medical Center, Dana 509, Harvard Medical School, 330 Brookline Ave, Boston, MA 02215, United States

Editorial office
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Clinical Cases
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
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Fax: +86-10-85381893
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In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/

ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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