Perineal reconstruction with biologic graft vulvoplasty for verrucous carcinoma treated by repeated vulvar excisions: a case report

ELVIRA BRĂTILĂ1, CORNEL PETRE BRĂTILĂ2, DIANA-ELENA COMANDĂȘU3, VASILICA BAȘIĆ3, DOINA MIHAELA POP4, VLAD DENIS CONSTANTIN5, MONICA MIHAELA CÎRSTOIU6, RUXANDRA STĂNCULESCU1

Abstract
Vulvar neoplasia represents 5% of malignancies in female genital tract and 0.6% of all cancers in women. Although it is known to be a rare type of cancer, which occurs especially in elderly women, its incidence is increasing in young females because of its association with the human papillomavirus (HPV). In this paper, we report the case of a 46-year-old woman, gravimetry 4, parity 3, with a medical history of multiple vulvar excisions for recurrent ulcerative vulvar lesions during a period of 11 years. The first lesion appeared in 2003, it was excised and the histopathological result showed squamous cell carcinoma with undifferentiated areas and chronic ulcerative inflammation. The patient underwent radiation therapy remaining at the end of it a small-ulcerated lesion at the superior vulvar commissure, which was biopsied in 2004 showing chronic ulcerative inflammation with reparatory areas of squamous immature benign metaplasia. In April 2014, a dermatological consult described vulvar scleroatrophic lichen confirmed by a biopsy. In November 2014, the patient presented to our clinic when a vicious vulvar scar was detected, with a transformed tegument with aspect of atrophic lichen. A perineal reconstruction including anal sphincter plasty was performed. Due to the important remaining skin defect, a Surgisus graft vulvoplasty was performed. The histopathological result of the excised suspect areas was vulvar intraepithelial high-grade neoplasia (VIN III). A retrospective histopathological review of the case established that is more accurate to consider that the vulvar lesions were, all along, a very well differentiated squamous cell carcinoma (verrucous carcinoma), which lacks cytopathic effect of HPV infection, has a low p53 expression but a high Ki67. Case evolution was favorable with the acceptance and integration of the biologic grafts at two months after surgery and normal healing.

Keywords: vulvar cancer, squamous cell carcinoma, vulvoplasty, perineal reconstruction, biologic graft.

Introduction
Vulvar neoplasia represents 5% of malignancies in female genital tract and 0.6% of all cancers in women. Although it is known to be a rare type of cancer, which occurs especially in elderly women, its incidence is increasing in young females because of its association with the human papillomavirus (HPV). Approximately 90% of vulvar cancer is squamous cell carcinoma, which originates from epidermal squamous cells, the most common type of skin cell [1]. In 5% of the cases, the cancer is present at multiple locations [1]. The National Cancer Institute from the United States of America has reported that vulvar cancer is one of the 12 malignancies whose incidence has recently increased [2]. This type of neoplasia has usually a slow development and may be preceded by a precancerous condition referred to as vulvar intraepithelial neoplasia (VIN) or dysplasia, which can lead to in situ carcinoma and then invasive carcinoma [3]. Although we cannot identify the exact cause of vulvar cancer, certain factors seem to increase the risk including: increased age, HPV as a sexually-transmitted infection that increases the risk of several cancers, including vulvar and cervical cancer, smoking, chronic immunodeficiency caused by human immunodeficiency virus (HIV) infection, which makes patients more susceptible to HPV infections, herpes simplex virus 2 infection, vulvar intraepithelial neoplasia as a precancerous condition, lichen sclerosus, which causes the vulvar skin to become thin and itchy, chronic vulvar inflammation [4, 5].

Vulvar verrucous carcinoma represents a particular type of vulvar neoplasia, which is characterized by slow evolution, rarely metastasizing to the lymph nodes appears as an exophytic tumor that is frequently locally destructive. The incidence of this type of malignancy is about 1–2%
of all gynecological cancers. Vulvar cancers that are HPV positive have a better prognosis than those that are HPV negative [6].

In this paper, we report a case of a 46-year-old woman that underwent multiple surgical vulvar excisions for vulvar recurrent lesions, which lead to an extensive scarring of the area and needed perineal reconstruction using a biologic graft. The case is unique due to the young age of the patient at the moment of the appearance of the vulvar cancer, the long history of reappearing vulvar ulcerations totaling 11 years and the impossibility to preserve a skin graft from the patient caused by a gaseous gangrene on the left leg that was treated by double fasciomy and produced extensive scarring.

Case report

The 46-year-old Caucasian woman, gravity 4, parity 3, underwent multiple vulvar surgical excisions for different types of lesions, from squamous cell carcinoma to condyloma and scleratrophic lichen. The recurrent lesions and their treatments extended on a long period of eleven years. During this time, the psychological effect of the vulvar scarring was manifested more and more pronounced. Informed consent of the patient was obtained.

The medical history of the patient revealed chronic anemia and chronic thrombocytosis, the peripheral smear showing marked anisocytosis, poikilocytosis, target erythrocytes with micro- and macro-platelets, for which she received preoperative intravenous iron supplements. An important aspect of her medical history is represented by an accidental foot wound caused by a metal part 18 years prior to her presentation, that rapidly caused a spreading infection, which turned out to be gaseous gangrene of the entire left leg, and needed double fasciomy on her thigh and shank. This potentially fatal infection leads to extensive scarring and venous circulation dysfunctions.

The first vulvar lesion appeared in 2003 and was located on the right major labia and lateral to the clitoris, with a diameter of 1.5 cm. It was excised and the histopathological result showed squamous cell carcinoma with undifferentiated areas and chronic ulcerative inflammation (pT1aMxNx) (Figure 1).

The patient underwent radiation therapy (cobalt therapy) for five days a week, during six weeks after surgery, remaining at the end of it a small remaining ulcerated lesion at the superior vulvar commissure, which was biopsied in 2004 showing chronic ulcerative inflammation with reparatory areas of squamous immature benign metaplasia.

One year later another tumor appeared with rough aspect and a size of 3/1 cm appeared on the left major labia and was excised, with the histopathological result of acanthosis and hyperkeratosis papilloma. Topical anti-inflammatory and healing products and systemic nonspecific immunomodulatory agents were used as adjuvants.

Three years after that, a right inguinal lymphadenopathy appeared and was excised in order to establish whether it is a metastatic adenopathy or a reactive infectious one. The pathological report described lymph nodes with multiple formations formed by areas of epithelioid multinucleated giant cells and inflammatory elements, the aspect advocating for benign granulomatosis, either sarcoidosis, or an infectious originated disease like histoplasmosis or toxoplasmosis. More tests including immunohistochemistry and serology were recommended. One year later, a left microadenopathy was detected and treated with a nonspecific immunomodulatory agent.

In November 2011, a follow-up consult revealed a grossly endured area on the posterior perineum and at the posterior labial commissure with a small erosion at the anterior vulvar commissure and erythematous areas on the former right labia. A partial posterior vulvectomy was performed in January 2012, and the histopathological report of the piece showed tissue fragments lined by squamous papillomatous epithelium with marked acanthosis and hyperkeratosis with abundant inflammatory subepithelial hyperplasmoctytaire infiltrate accompanied by stromal fibrosis with chronic granulomatous reaction with giant foreign body cells and nodular formation composed of hyalinized fiber bundles with micropolcalifications. The conclusion of the report was chronic granulomatous inflammation with giant foreign body cells and lichen planus with hyperkeratosis.

In September 2013, a check-up found an open vulva and a small tumor of 12 mm on the left scarred vulva and an anterior vaginal mucosa tumor of 2/1 cm. Their electro-ressection was performed, the histopathological report describing fibrous connective tissue wrapped by multi-layered squamous epithelium with acanthosis, marked hyperkeratosis and papillomatosis and presence of HPV-type cytoplasmic and nucleus changes with the result of vaginal condyloma (low-grade squamous intraepithelial Neoplasia) and vulvar condylomatosus. Immunohistochemistry showed an aggressive pattern with Ki67 and P16 positive. HPV genotyping confirmed the infection with two low risk strains: 11 and 73 and she had the HPV three shots vaccine.

On a closer look when reexamining the slides and retrospectively considering the entire history of the patient, the accurate histopathological result for this biopsy piece is verrucous carcinoma, the hyperkeratotic undulating warty bulbous epithelial pegs being suggestive histological features for this diagnosis (Figures 2-4).

In April 2014, vulvar scleratrophic lichen was confirmed by a biopsy and followed by cauteryization, the histological result showing squamous mucosa fragments with moderate hyperkeratosis, acanthosis and papillomatosis; in the upper layers rare keratinocytes with perinuclear halo, vascular capillaries dilatations and moderate lympho-monocytic inflammatory infiltrate with rare dermal eosinophils and areas of collagenous homogenization in the superficial dermis.

In November 2014, the patient presented to us accusing a vicious both psychological and physical invalidating vulvar scar due to the multiple surgical interventions and excisions she had suffered in the area.

Gynecologic exam detected vicious vulvar scar, with a transformed tegument on the posterior perineum, the left major labia, an endured area lateral to the right from the urethral external orifice, with aspect of atrophic lichen, the multiple excisions causing a 3rd degree perineal tear.

A perineal reconstruction including anal sphincter plasty was performed. Due to the important remaining skin defect that could not be covered with rotated skin flaps
because of the lack of elasticity of the tissues a Surgisis graft vulvoplasty was performed. Another Surgisis graft was used to replace the abnormal skin area of the left labia. Case evolution was favorable with the integration of the biologic grafts at two months after surgery and healing within expected terms.

The histopathological result of the excised suspect areas showed an extensive range of acanthosis and hyperkeratosis lesions and intraepithelial proliferation compatible with vulvar intraepithelial high-grade neoplasia (VIN III), while the perilesional dermis was hyperemic, edematous with marked chronic inflammatory infiltrate. The immunohistochemistry performed described the lesions as being cytokeratin (CK) 34βE12 positive in the squamous epithelium and Ki67 diffusely positive (10% in the suprabasal epidermis and 50–60% in the squamous cells in the VIN lesion area) (Figures 5 and 6).

In the basal and suprabasal epidermis p53 immunostaining was diffusely (10%) positive (Figure 7). Although p16 is considered a transformant HPV infection marker, in our case the tumor was p16 negative (Figure 8).

Stratified squamous epithelium of the vulva is commonly evaluated with high molecular weight cytokeratins as CK 34βE12, low molecular weight cytokeratins (CAM 5.2) being negative. In this particular case, CK 34βE12 was meant to identify small foci of invasive squamous carcinoma, not easily spotted in routine histological sections mainly due to inflammatory stromal reaction.

The Ki67 antigen is a nuclear non-histone protein present in all stages of cell cycle except G0. It is identified with an anti Ki67 antibody in normal and pathological tissues. The percentage of positive cells and the specific location have importance. In VIN (vulval intraepithelial neoplasia), VAIN (vaginal intraepithelial neoplasia), CIN (cervical intraepithelial neoplasia) and squamous carcinoma positive cells progressively increase in height within the epithelium with increasing lesion grade.

As already stated, in our particular case, p16 is a surrogate marker for the presence of HPV. Altered Ki67 antigen expression without p16 support a non-related HPV lesion.

Mutations and overexpression of p53 gene are very common in many types of tumors. Since p53 is also known to play a role as a marker for dysplasia, we found it useful in conjunction with Ki67 to establish the level of dysplasia. D-VIN III usually express p53 strongly and diffusely only in basal and suprabasal epithelium, as in our case, whereas p53 immunoreactivity is weak and focal or totally negative in squamous hyperplasia and lichen sclerosus.

**Figure 1** – Well-differentiated squamous cell carcinoma with minimal stromal invasion and inflammatory infiltrate. HE staining, ×100.

**Figure 2** – Hyperkeratotic undulating warty bulbous epithelial pegs suggesting verrucous carcinoma. HE staining, ×100.

**Figure 3** – Verrucous carcinoma, histopathological appearance overall. HE staining, ×100.

**Figure 4** – Inflammatory infiltrate – detail of previous figure. HE staining, ×200.
The immunohistochemical profile of the squamous lesions analyzed in this particular case, using the combined values for the already mentioned antibodies, leads us to the conclusion that this is mainly a differentiated VIN III lesion associated with minimally invasive verrucous carcinoma.

Discussion

Vulvar cancerous and precancerous lesions represent a borderline pathology between dermatology and gynecology. The gynecologist’s main concern is VIN (vulvar intraepithelial neoplasia) whose recent classification has a major clinical interest. Classification of squamous vulvar precancerous lesions includes a three degrees of severity of dysplastic changes (VIN I, II and III). According to the histological features, VIN has been subdivided in 2004 by Society for Study of Vulvovaginal Disease (ISSVD) into the usual VIN (u-VIN) and differentiated VIN (d-VIN), which are basically the two pathogenic pathways of vulvar squamous cell carcinoma [6]. U-VIN is associated with the human papillomavirus infection and is the vulvar correspondent of cervical intraepithelial neoplasia, occurring in young women with a history of cervical, vaginal or vulvar premalignant lesions. D-VIN is associated with lichen sclerosus and chronic lichen simplex and frequently affects postmenopausal women without any history of other dysplastic genital lesions. D-VIN usually has a higher stromal invasiveness than u-VIN, its malignant potential is analogous to carcinoma in situ (VIN III). The histological characteristics of d-VIN are subtle including basal atypia while the superficial layers of the squamous epithelium remain well differentiated, making it easy to be confused with u-VIN I, lichen sclerosus and chronic lichen simplex in vulvar biopsies [7]. In our case, the exact type and therefore the etiology of the squamous cell carcinoma is difficult to identify. Both HPV infection confirmed by the nucleus and cytoplasmic transformation induced by the virus and by the genotyping that identified low risk strains 11 and 73, and lichenoid transformation detected on the biopsies that showed vulvar scleroatrophic lichen could represent the starting point of the neoplasia. The age of the patient at the debut of the symptomatology was relatively young (35 years), pleaded for the viral etiology, while the repeated normal Pap smear results, the histopathological aspect of extended lichen and the
local relatively good therapeutic response to topical corticoids incline towards d-VIN.

Although in the specialty literature the incidence of vulvar cancer has been reported stable or only minimally increasing, the results of a retrospective study in 2008 show that the number of patients with invasive vulvar cancer has doubled in the last three decades in Germany, with a nearly four-fold increase in younger patients because of HPV high-risk strains infection [8]. The location of the tumor changed from the labia to the area between the clitoris and urethra. That is why the study strongly recommends widely implemented prophylactic HPV vaccination, proven highly effective against anogenital disease, hoping to be an important contribution factor to the reduction of the risk of vulvar carcinomas in younger women [8, 9].

Even if the incidence of precancerous vulvar lesions appears to be rising in the younger population, vulvar invasive squamous cell carcinoma is still extremely rare [10]. That is why diagnostic biopsy is often delayed and ablative therapy procedures often instituted without a correct histological diagnosis. Awareness of the possibility of invasive vulvar carcinoma, even in the relatively young patient, should lead to prompt histological evaluation of all vulvar lesions, especially when ablation of extensive lesions is planned [11]. This management of vulvar lesions not only brings a correct diagnosis, which leads to an optimal treatment, but also minimizes the risk of recurrences, which in our case were numerous [12].

Retrospectively reviewing the case and reexamining the histopathological aspects of the multiple biopsies along the eleven years of relapses, it is probably more accurate to consider that the recurrent vulvar lesions were, all along, a very well differentiated squamous cell carcinoma (verrucous carcinoma), which lacks cytopathic effect of HPV infection, has a low p53 expression but a high Ki67 expression. Verrucous carcinoma is a type of malignancy very difficult to diagnose by simple biopsy because is very well differentiated, which usually needs a larger excision area in order to establish an accurate diagnostic. The difficulty of the diagnostic in our case resided in the analysis of multiple small tissue fragments, from different areas of the vulvar region, in different moments of the disease’s evolution and analyzed by different pathologists, who did not have the entire history of the patient. Putting together all the histopathological characteristics and carefully reexamining the excision samples from 2003 until 2014, along with the patient’s history and evolution, allowed us to diagnose a rare case of verrucous vulvar carcinoma, misread until now. Verrucous carcinoma of the vulva is a rare type of squamous cell vulvar carcinoma, constituting less than 1% of vulvar cancer overall. The etiology of verrucous carcinoma is not known [13]. It is considered that vulvar cancer occurs especially in women with primary cancer located elsewhere in the genital tract, especially in the cervix [14]. Verrucous carcinoma can be found also in the oropharynx, perianal region, cervix, vagina, penis, scrotum, bladder, and anorectal region [15]. The diagnosis is usually difficult to perform, that is why it is indicated to practice large excisions, in order to avoid misdiagnosis and inadequate treatment. Verrucous carcinoma has a series of histological diagnostic criteria such as a “pushing” tumor–dermal interface with minimal stroma between the acanthotic epithelium, minimal nuclear atypia, hyperkeratotic areas on the surface of the tumor with little keratin formation inside the tumor, and diffuse chronic inflammation of the stroma [16]. It distinguishes from condyloma acuminata by this broad papillae and the absence of koilocytosis. It differs from the more common types of squamous cell carcinoma because it shows no more than minimal nuclear atypia and does not have infiltrative growth.

Verrucous carcinomas are locally invasive, it has a tendency to reappear locally after initial wide excision, but do not metastasize [17, 18]. It appears frequently in elderly postmenopausal women, but during the last years an increase in the incidence of this tumor in younger women has been observed [19, 20]. Women with verrucous carcinoma refer to the specialist because of the presence of a mass in the external genitalia usually itching and sometimes painful. Histologically, it presents minimal cellular atypia and very mitotic figures compared with well-differentiated squamous cell carcinoma. Verrucous carcinoma of the vulva may be difficult to treat. The scientific community supports the fact that a wide local excision should be the best treatment, because of the lack of spontaneous metastasis. Relapses have been described after radiotherapy, because verrucous carcinoma is resistant to radiotherapy and sometimes may undergo transformation to squamous cell carcinoma [21]. Local application of podophyllin, bleomycin therapy, and cryosurgery are ineffective methods of treatment. In fact, the treatment is still a matter of discussion. It is very important to perform an extensive excision of the ill-defined disease, because of the potential invasion of deep surrouding tissues. In some cases, radiation may cause anaplastic transformation, not recognized yet by all physicians. The prognosis of verrucous carcinoma is in generally good if large excision is being performed [22].

The uniqueness of the case resides in the long period of recurrent appearance of vulvar lesions followed by multiple excisions, extended on a period of 11 years, which culminated in 2012 with a partial posterior vulvectomy. These surgical interventions affected greatly the vulvar anatomy and function, also having an important psychological and sexual impact. Choosing the optimal surgical treatment for women suffering from vulvar precancerous or cancerous lesions is balancing the need to maintain sexual function with the necessity to obtain oncological free resection margins [23]. Until recent, the surgical interventions for vulvar cancer included excising a large amount of surrounding normal tissue and often local lymph nodes, regardless of the stage of cancer. This type of extensive surgery has a good prognosis, but is deforming and impairs the woman’s sexual activity, especially if the clitoris has been affected or removed entirely. Nowadays, the quality of life and sexuality represents a necessity. In early detected cases, a limited excision is enough, and the sentinel node biopsy is an alternative procedure [24]. A study published in 1992 by Kelley et al. suggests that vulvar squamous cell carcinoma with 1 mm or less of stromal invasion can be treated by local resection alone, without inguinal node dissection [25]. They did not find...
any statistically significant associations between the reappearance of cancer and age, symptom duration, margin status, location, FIGO stage or coexisting VIN. The study states that coexisting dysplasia and relatively variable aspects makes the application of FIGO staging criteria very difficult in lesions with minimal focal invasion. It recommends wide excision for high-risk VIN lesions and if final pathologic report proves deeper invasion, a selective lymph node dissection as a second procedure followed by colposcopic surveillance and biopsies in these patients.

For our patient, the extensive scarring was the reason she presented to us. Her vulva had developed a vicious scar due to the multiple excisions suffered. Because of the absence of the major labia that had been removed and the posterior partial vulvectomy suffered, the local aspect showed on inspection a third degree perineal tear, including thus the involvement of external anal sphincter.

Reconstruction of the severely scarred vulva could have been realized by multiple techniques, according to the local situation, the extent of the defect, the possibility to remove an autograft from the patient and the experience of the surgeon. The reconstructive surgical technique was meant to realize a total perineal reconstruction, including anal sphincter plasty. Because of the important remaining skin defect that could not be covered with rotated flaps due to the weak elasticity of the tissues a Surgisis graft vulvoplasty was performed. A second biologic graft was used to substitute the vicious scar and the lichenoid transformed tegument that replaced the left labia. A biologic Surgisis graft was preferred to the alternative of harvesting skin graft from another region of the patient and using it in the vulvar area, because of the patient’s history of gaseous gangrene on the entire left leg that suffered scar due to the multiple excisions suffered. Because of the important remaining skin defect that could not be covered with rotated flaps due to the weak elasticity of the tissues a Surgisis graft vulvoplasty was performed. A second biologic graft was used to substitute the vicious scar and the lichenoid transformed tegument that replaced the left labia. A biologic Surgisis graft was preferred to the alternative of harvesting skin graft from another region of the patient and using it in the vulvar area, because of the patient’s history of gaseous gangrene on the entire left leg that suffered scar due to the multiple excisions suffered.

Conclusions
The presented case emphasizes the importance of conservatory treatment in cases that are suitable, especially in locations like the vulva, with numerous psychosocial implications. Maintaining the function and anatomy of a cancer site should be a desideratum in the modern surgery, in order to preserve a woman’s quality of life after the oncological treatment. Reconstructive techniques are more and more widespread and they should be individualized for every patient according to the local status, the technical possibilities but also the patient’s desires. Perineal reconstruction using biologic grafts is a surgical option for the cases when extensive scarring impairs classical plasty using rotated skin flaps due to a major defect.

Conflict of interests
The authors declare that they have no conflict of interests.

Acknowledgments
This work received financial support through the project entitled “CERO – Career profile: Romanian Researcher”, Grant Number POSDRU/159/1.5/S/135760, cofinanced by the European Social Fund for Sectoral Operational Programme Human Resources Development 2007–2013.

References
Corresponding author
Elvira Brătilă, University Assistant, MD, PhD, Department of Obstetrics and Gynecology, “Carol Davila” University of Medicine and Pharmacy, “St. Pantelimon” Emergency Clinical Hospital, 340–342 Pantelimon Avenue, 021659 Bucharest, Romania; Phone +40721–332 199, e-mail: elvirabarbulea@gmail.com

Received: November 22, 2014

Accepted: June 10, 2015