VERSITA

Cent. Eur. J. Med. • 4(1) • 2009 • 54-58
DOI: 10.2478/s11536-009-0016-7

Central European Journal of **Medicine**

Uterine leiomyoma with massive lymphoid infiltrate after colon cancer chemotherapy: an immunohistochemical investigation with special reference to lysosome-associated membrane protein expression

Research Article

Veselin T. Belovejdov[1*], Dorian I. Dikov[2], Marie L. Auriault[2], Christiane Copie-Bergman[3], Victoria S. Sarafian[4]

[1] *Department of General and Clinical Pathology, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria*

[2] *Service d'Anatomie et de Cytologie Pathologiques, Centre Hospitalier de Lagny-Marne La-Vallée, 77405 Lagny-Sur-Marne,France*

[3] *Département de Pathologie, Hôpital Henri Mondor, 94010 Créteil, France*

[4] *Department of Biology, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria*

Received 31 August 2008; Accepted 12 December 2008

Abstract: Uterine leiomyoma with massive lymphoid infiltration is a rare morphologic phenomenon. We describe the first case of uterine leiomyoma with lymphoid infiltration observed in a patient after chemotherapy for sigmoid cancer. We performed immunohistochemical analysis with a panel of antibodies to several markers. Detection of CD20, CD3, Ki67, CD68 and Epstein-Barr virus nuclear antigen assisted in the differential diagnosis and partial elucidation of the pathogenesis. In addition, we examined the lysosome-associated membrane proteins LAMP-1 and LAMP-2 for the first time in this lesion. Their expression was elevated, indicating enhanced autophagy, an indirect sign of degenerative changes in this benign tumor characterized by massive lymphoid infiltration.

Keywords: *Uterine leiomyoma with massive lymphoid infiltrate • Colon cancer • Immunohistochemistry • LAMPs*

1. Introduction

Uterine leiomyoma with massive lymphoid infiltration is an extremely rare phenomenon and its pathogenesis remains undefined. Only 16 cases have been reported to date [1-6]. Reactive alterations due to intrauterine pessars, hormonal therapy, or immune response dysregulation are thought to play basic role in the etiology and mechanism of this process.

Here, we present the first description of a leiomyoma with massive lymphoid infiltration observed in a patient with sigmoid cancer after surgery and subsequent chemotherapy. The leiomyoma was studied immunohistochemically using a large antibody panel including antibodies to CD20, CD3, Ki67, CD68, and Epstein-Barr virus (EBV) nuclear antigen. These markers aided in the differential diagnosis.

* *E-mail: vesbel@abv.bg*

2. Material and Methods

A 50-year-old woman presented with a 5-month history of metrorrhagia and enlargement of the uterus. The patient has had three previous pregnancies (one normal delivery, two induced abortions, and no missed abortion). She had history of sigmoid cancer stage C, operated 4 years before and had received adjuvant chemotherapy with 5-fluorouracil (5 FU; two successive treatments for 2 days within an interval of two weeks). The protocol included three consecutive infusions: folic acid 200 mg/m^2 for 120 min; 5 FU 400 mg for 10 min (bolus); and 5 FU 600 mg for 22 h. At the time of examination, the patient was in full remission, with normal serum level of tumor markers (CEA, 0.5 µg/ml; CA 19.9, 0.1 U/ml) and a normal blood cell count. The clinical examination and imaging analysis of internal organs revealed no pathological alterations. Abdomino-pelvic ultrasonography showed a uterine leiomyoma measuring 6 cm in its greatest diameter and a left-side hydro-salpinx. The patient was not climacteric and was using an intrauterine contraceptive device with progesterone. There was no history of vasculitis or of any connective tissue disease. She underwent total vaginal hysterectomy. She was healthy after the operation, and there was no recurrence at the latest follow-up, 11 months after the operation.

The surgical specimen was fixed in 10% neutral formalin, routinely processed and embedded in paraffin. The 4-µm thick sections were used for hematoxylin-phloxine-saffron/HPS/ and immunohistochemica stains. Immunohistochemistry was performed using the streptavidin-biotin peroxidase method (LSAB kit; Dako Corp., Carpinteria, CA, USA). Antibodies to the following proteins were used: CD3, CD20, CD68, Ki67, EBV nuclear antigen, smooth muscle actin (SMA), estrogen receptor (ER), progesterone receptor (PR), LAMP-1, and LAMP-2. The antibodies to the LAMP proteins were from Iowa University Hybridoma Bank (Iowa, USA), all others from DAKO.

3. Results

3.1. Macroscopic

Gross examination revealed that the surgical specimen weighed 245 g and consisted of: a fragment of uterine cervix (7 x 2 cm), a fragment of the uterine corpus (6 x 5 x 4 cm), and a separate well-circumscribed nodule measuring 7 x 6 x 3 cm. The tumor was relatively soft and lobulated, and the cut surface was white-yellow, with discrete fibrillarity (Figure 1).

Figure 1. Macroscopic appearance of the leiomyoma nodule.

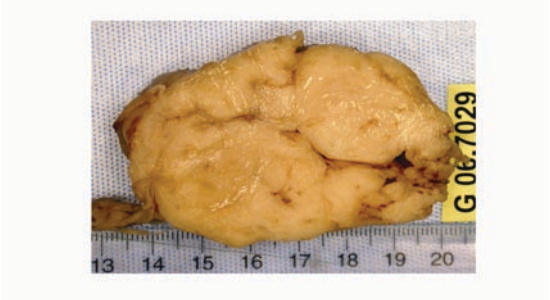
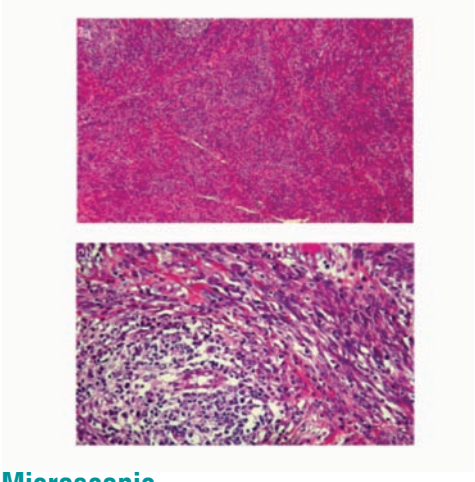


Figure 2. Hematoxylin-phloxine-saffron staining. Intramyomic lymphoid infiltration (a) (original magnification x 100). Lymphocytic concentration around a blood vessel and a lymph follicle formation (arrow) (b) (original magnification x 200).

a

b



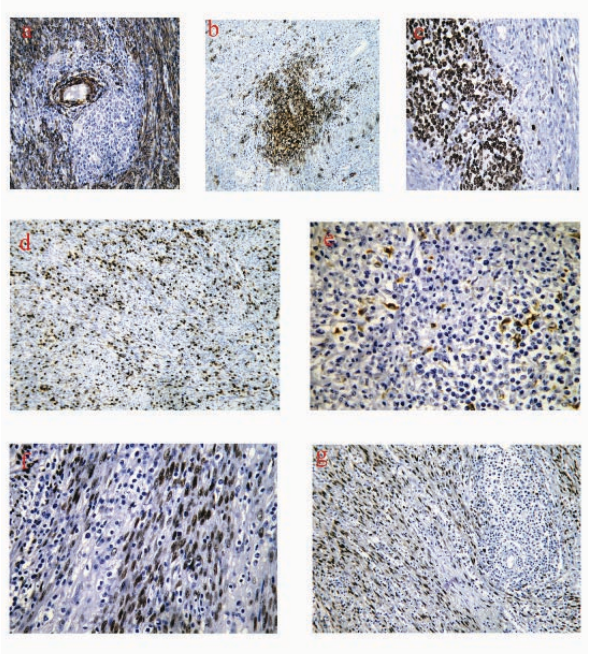
3.2. Microscopic

Proliferative endometrium. A nodular formation consisting of interlacing fascicles of spindle-shaped ovoid cells with lightly stained nuclei containing a single nucleolus was observed. Amongst the ovoid cells, a considerable number of diffusely scattered lymphocytes were detected, as well as single plasmocytes and macrophages. Lymphoblasts grouped in follicles were also noticed. The inflammatory infiltrate was restricted within the leiomyoma and did not penetrate into the surrounding myometrium. No necrosis, nuclear polymorphism, interstitial fibrosis, hyalinosis or vascular abnormalities were observed (HPS stain; Figure 2).

3.3. Immunohistochemistry

The spindle-shaped cells were stained strongly for smooth muscle actin and estrogen and progesterone receptors. Lymphoid cells in follicular zones were intensely positive for CD20, the central areas of the follicles showed a strong signal for Ki67 that diminished at the periphery and was negative in the smooth muscle cells. Almost all lymphocytes were located outside the follicles and those dispersed amongst leiomyocytes were

Figure 3. Immunohistochemistry. Intensive reaction for SMA in leiomyocytes and in perivascular smooth muscle cells within the lymphoid follicles (a) (original magnification x 100). Cells in the follicles are stained for Ki 67 (b) (original magnification x 100) and CD 20 (c) (original magnifications x 200). A lot of CD3+ cells (d) and few CD68+ cells (e) are found amongst smooth muscle cells outside the follicles (original magnifications x 100). Leiomyocytes are also positive for estrogen receptors (f), and progesterone receptors (g) (original magnifications x 200).



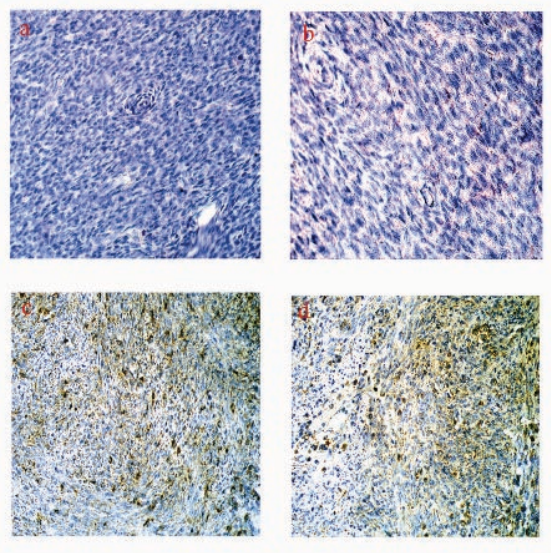
CD3-positive. Few histiocytes (CD68+) were detected (Figure 3). No immunoreactivity for EBV nuclear antigen was observed (data not shown).

The majority of smooth muscle cells were immunopositive for LAMP-1 and LAMP-2, with the expression of LAMP-2 being more prominent. Both markers were detected, with higher intensity in the leiomyoma sample with lymphoid infiltration than in the control sample without lymphoid infiltration (examined in parallel) obtained from a woman of the same age without an intrauterine pessar (Figure 4).

4. Discussion

Intramyomic lymphoid infiltration (IMLI) is a rare morphologic phenomenon [1,5]. Such a histopathologic finding necessitates a differential diagnosis between a benign smooth muscle tumor and a lymphoma, granulocytic sarcoma, pyomyoma, and inflammatory pseudotumor [1,5], all of which are characterized by lymphocytic infiltration. However, B-cell lymphomas affect mainly the uterine cervix rather than the myometrium. The large neoplastic lymphocytes in lymphomas differ

Figure 4. Immunohistochemistry. Expression of LAMP-1 (a) (original magnification x 100) and LAMP-2 (b) (original magnification x 200) in the control leiomyoma nodule. Expression of LAMP-1 (c) and LAMP-2 (d) (original magnifications x 200) in the leiomyoma with lymphoid infiltration.



from the small, normal mature ones and diffusely infiltrate the uterine muscular layers. In most cases of leiomyoma reported to date, including the present one, the lymphocytic infiltration is localized within the myoma and is composed of morphologically normal cells only [3,5,6]. Granulocytic sarcoma is characterized by blast cells from the myeloid lineage [5], which are not typical of IMLI.

Pyomyoma is observed mainly in younger women after delivery. The infiltrate is composed not only of lymphocytes, but also of neutrophils, bacterial colonies and pus, which penetrate quite frequently into the adjacent myometrium [5,6]. Aside from lymphocytes, the inflammatory pseudotumor also contains many fibroblasts, myofibroblasts, macrophages, and several granulocytes. Quite often, surrounding tissues are also involved in the process [1,7].

The first paper on IMLI by Ferry *et al.* [4] regards it as a reactive process. This is based on the fact that in some cases (as it is in ours) an intrauterine pessar is present. In our sample, we excluded previous EBV infection as a causative agent for the subsequent lymphocytic attack because of the negative reaction for EBV nuclear antigen.

Recent publications suggest an association between IMLI and the preoperative hormonal therapy with gonadotropin-releasing hormone antagonists, which leads to low levels of circulating sex hormones [7-9]. Thus, the decreased estrogen effect results in reduction of the uterine corpus size and, basically, of

the leiomyoma itself. Such an outcome is observed also in leiomyomas without lymphoid infiltration treated with equivalent therapy. Besides a reduction of size, increased cellularity, necrosis, decreased cell volume, atrophy, diminished microfilaments, mitochondrial swelling, pyknosis, and cellular deformity are also reported [10-13]. In leiomyomas without lymphoid infiltration, modulation of the reactivity of progesterone and estrogen receptors is detected as well [14]. In our case, the alterations were observed mainly in the reactivity of leiomyocytes for LAMPs. These changes might be due to the different treatment, namely, with chemotherapy instead with hormones. This results in preserved level of estrogen and progesterone receptors, as well as a lack of cellular atypism or increased mitotic activity. In our case, the findings described clearly result from the chemotherapy directed against the sigmoid carcinoma. This is supported by the fact that 5 FU produces growth inhibition and apoptotic cell death in another type of uterine tumor, uterine carcinoma [15]. As a consequence, the treatment affects all tumor tissues, including the leiomyoma.

Another hypothesis is that there is a link between IMLI and an autoimmune process provoked by the hormone-induced changes in the leiomyoma [1,16]. The existence of a possible immunopathological reaction is supported by the immunohistochemical profile of lymphocytes [3,5]. We assume that the chemotherapy used in our case to treat the colon carcinoma might have suppressed the proliferative activity of leiomyomic cells as well. Although the cytostatic treatment restrains the immune response, it could not be excluded that the lymphocytic attack within the leiomyoma might have resulted from the dysregulated immune status. Yoshimatsu *et al.* [17] also assumed that adjuvant chemotherapy with 5 FU in colorectal carcinoma has a negative effect on host immunity.

The positive staining of leiomyocytes for LAMP-1 and LAMP-2 helps clarify the pathogenesis of IMLI. These glycoproteins are major constituents of lysosomal membranes. The enhanced expression of LAMPs in the leiomyoma studied compared to the control sample indicates the activation of autophagy. On the other hand, the enhanced reactivity for LAMP-2 compared to LAMP-1 supports the idea that autophagic degradation occurs, as Cuervo and Dice [18] showed that the level of the LAMP-2a isoform in lysosomal membranes of rat fibroblasts directly correlates with the rate of chaperone-mediated autophagy in a variety of physiological and pathological conditions.

Our previous investigation indicates the possible participation of LAMP in the process of autophagy in granulation tissue, a reparative process eliminating damaged or degenerated cells [19]. Autophagy is regarded as a process typical for structures composed of cells dependent on hormonal stimuli [20] such as leiomyomas. The treatment either with hormones (including a hormonal pessar) or cytostatic drugs, as in our case, might cause different effects including degenerative alterations that could trigger autophagy. The degeneration and the biochemical and morphological characteristics of this type of cell death (*i.e.*, structure of lysosomes and their content) could direct a lymphocytic attack, which is quite unusual for these tumors.

In conclusion, we suspect that drug-induced degenerative changes (either by hormones or cytostatic therapy) of smooth muscle cells within the leiomyoma are the major reason for the development of intratumoral lymphocytic infiltration. This infiltration usually occurs in the background of already dysregulated immune function, and the degenerative process results in enhanced autophagy. Autophagy could reflect the benign nature of IMLI because it is known to act as a mechanism of tumor suppression [21]. Also, autophagy is known to be down-regulated during malignant transformation, and it is triggered in some cancer types by anticancer agents [20]. This process of autophagy appears to be rare in most malignancies.

References

[1] Paik S.S., Oh Y.H., Jang K.S., Han H.X., Cho S.H., Uterine leiomyoma with massive lymphoid infiltration: case report and review of the literature, Pathol. Int., 2004, 54, 343-348

[2] Wei S., Feng R., Cui Q., Luo Y., Zhang S., Uterine adenomyoma with lymphoid infiltration simulating lymphoma, Gynecol. Oncol. , 2004, 95, 409-411

[3] Saglam A., Guler G., Taskin M., Ayhan A., Uner A., Uterine leiomyoma with prominent lymphoid infiltrate, Int. J. Gynecol. Cancer, 2005, 15, 167–170

[4] Ferry J.A., Harris N.L., Scully R.E., Uterine leiomyomas with lymphoid infiltration simulating lymphoma. A report of seven cases, Int. J. Gynecol. Pathol., 1989, 8, 263-270

[5] Chuang S.S., Lin C.N., Li C.Y., Wu C.H., Uterine leiomyoma with massive lymphocytic infiltration simulating malignant lymphoma. A case report with immunohistochemical study showing that the infiltrating lymphocytes are cytotoxic T cells, Pathol. Res. Pract., 2001, 197, 135- 138

[6] Botsis D., Trakakis E., Kondis-Pafitis A., Kontoravdis A., Kassanos D., Chryssikopoulos A. et al. , Leiomyoma of the uterus with massive lymphoid infiltration simulating lymphoma. A case report, Eur. J. Gynaecol. Oncol., 1999, 20, 61-62

[7] McClean G., McCluggage W.G., Unusual morphologic features of uterine leiomyomas treated with gonadotropin-releasing hormone agonists: massive lymphoid infiltration and vasculitis, Int. J. Surg. Pathol., 2003, 11, 339-344

[8] Ohmori T., Wakamoto R., Lu L.M., Okada K., M. Nose M., Immunohistochemical study of a case of uterine leiomyoma showing massive lymphoid infiltration and localized vasculitis after LH-RH derivant treatment, Histopathology, 2002, 41, 276-277

[9] Bardseley V., Cooper P., Peat D., Massive lymphocytic infiltration of uterine leiomyomas associated with GnRN agonist treatment, Histopathology, 1998, 33, 80–83

[10] Crow J., Gardner R.L., McSweeney G., Shaw R.W., Morphological changes in uterine leiomyomas treated by GnRH agonist goserelin, Int. J. Gynecol. Pathol., 1995, 14, 235-242

[11] Resta L., Sanguedolce F., Orsini, G., Laricchia L., Piscitelli D., Fiore M.G., Morphometric and histological evaluation of uterine leiomyomas treated with GnRH agonists or progestational agents, Pathologica, 2004, 96, 35-41

[12] Colgan T.J., Pendergast S., LeBlanc M., The histopathology of uterine leiomyomas following treatment with gonadotropin-releasing hormone analogues, Hum. Pathol., 1993, 24, 1073-1077

[13] Ito F., Kawamura N., Ichimura T., Tsujimura A., Ishiko O., Ogita S., Ultrastructural comparison of uterine leiomyoma cells from the same myoma nodule before and after gonadotropin-releasing hormone agonist treatment, Fertil. Steril. , 2001, 75, 125-130

[14] Bozzini N., Rodrigues C., Petti D., Bevilacqua R., Goncalves, S., Pinotti J., Efects of treatment with gonadotropin releasing hormone agonist on the uterine leiomyomata structure, Acta Obstet. Gynecol. Scan., 2003 82, 330-334

[15] Ueda M., Kumagai K., Ueki K., Inoki C., Orino I., Ueki M., Growth inhibition and apoptotic cell death in uterine cervical carcinoma cells induced by 5-fluorouracil, Int. J. Cancer, 1997, 71, 668-674

[16] Laforga J., Aranda F., Uterine leiomyoma with T-cell infiltration associated with GnRH agonist goserelin, Histopathology, 1999, 34, 471–472

[17] Yoshimatsu K., Kuhara K., Itagaki, H. Aizawa M., Yokomizo H., Fujimoto T., et al., Changes of immunological parameters reflect quality of life-related toxicity during chemotherapy in patients with advanced colorectal cancer, Anticancer Res., 2008, 21, 373-378

[18] Cuervo A.M., Dice J.F., Unique properties of lamp 2 compared to other lamp 2 isoforms, J. Cell. Sci., 2000, 24, 4441-4450

[19] Sarafian V., Dikov D., LAMPs and ABH histo-blood group antigens in granulation tissue, APMIS, 2007, 114, 701-706

[20] Gozuacik D., Kimchi A., Autophagy as a cell death and tumor suppressor mechanism, Oncogene, 2004, 23, 2891-2906

[21] Huang J., Klionsky D.J., Autophagy and human disease, Cell Cycle, 2007, 6, 1837-1849