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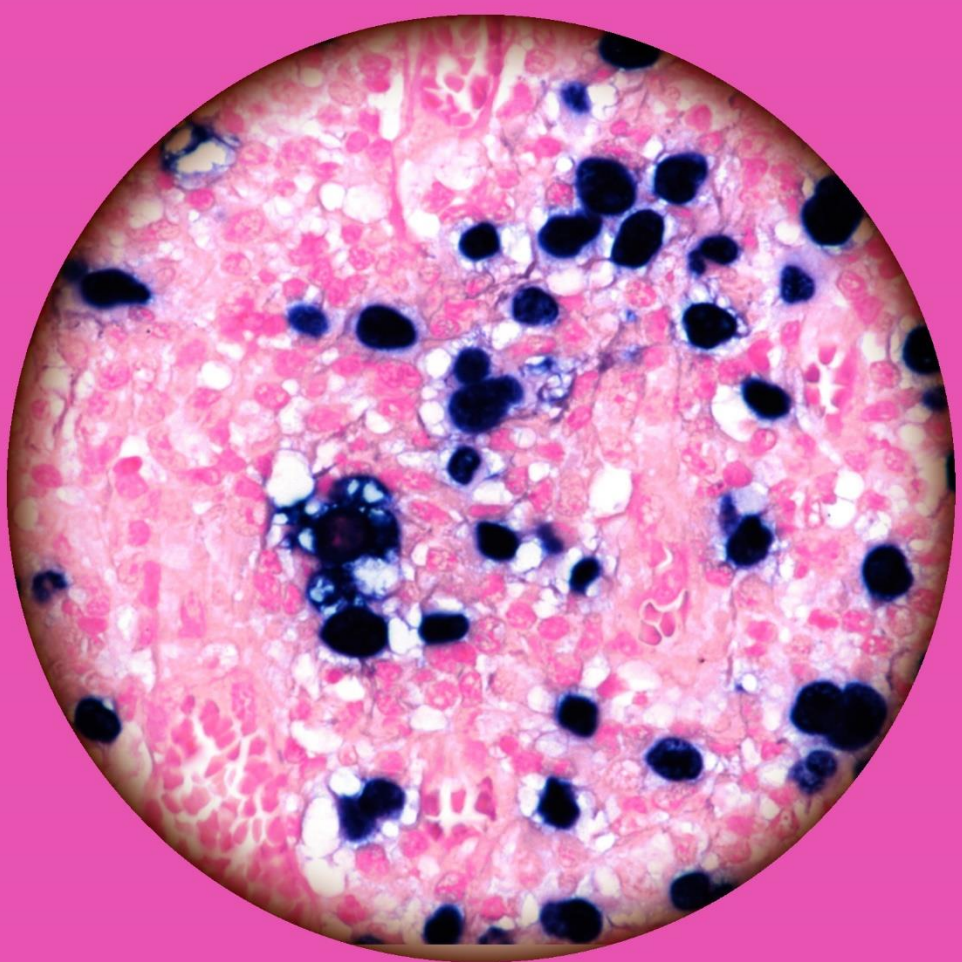

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Immunohistochemical Investigation of the role of Foxp3+ T regulatory cells in patients with Inflammatory Bowel Disease complicated with infectious intestinal presence of Cytomegalovirus

Despoina Antaraki [1], Georgios Kakiopoulos [2], Sophia Pappa[2], Aliki Liakea [3], Georgia-Heleni Thomopoulou[4], Eirini Thimara[3], Andreas C. Lazaris[3]

Abstract

Aim: We attempted to study whether the presence of T-regulatory cells in tissue obtained from patients with an inflammatory bowel disease augment when Cytomegalovirus coexists in the bowel. Experimental data were analysed using statistical methods and were combined with bibliographical references in an attempt to investigate the role of T-reg cells in immunodeficient patients with or without CMV infection.

Materials and Methods: Sixty-one cases of inflammatory bowel disease were divided into two groups. The first one included patients with either Crohn's disease or ulcerative colitis that co-existed with CMV bowel infection, whereas the second group (control group) consisted of patients with inflammatory bowel disease without CMV infection. Formalin-fixed, paraffin-embedded tissues were immunohistochemically elaborated with CMV-specific polyclonal antibodies and the stained sections were microscopically evaluated in order to define Foxp3 protein levels and the attendance of Tregs in specific tissues.

Results: Statistical analysis of the evaluated samples revealed statistically significant correlation between CMV's presence and the number of Tregs in bowel tissue. There was no evidence that CMV is related with acute phases of inflammatory bowel disease. Tregs were diminished in patients with disease in recession. Eosinophils attendance was also examined and the results proved statistically significant correlation between the number of eosinophils and Tregs number in the examined samples.

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Conclusions: Our investigation provides strong evidence that the increase of Treg population in colon mucosa is probably an underlying defensive mechanism of the human organism in order to control and suppress the local inflammatory reaction caused by infectious factors in immune deficient patients. Further study could help to shed light on Tregs' suppressive potency and its potential value in clinical therapeutics.

Introduction

Regulatory T cells (Treg) develop mainly in the thymus gland (most specifically called natural Tregs –nTregs) and conduce to the development of responses to self antigens, allergens and commensal microbiota, as well as immune homeostasis to infectious factors and tumors. Various tissue-specific inflammatory responses depend on the quantity and correspondence of Tregs.[1] Function and proliferation of Treg cells can indirectly be measured by detecting forkhead box protein 3 (Foxp3), which is a transcriptional factor specific for regulatory T cells. Treg cells deficiency due to mutations of the FOXP3 gene leads to immune mediated inflammatory diseases. [2]

$CD4^+CD25^+Foxp3^+$ regulatory cells (Tregs) constitute 5-10% of $CD4^+$ T cells and produce various anti-inflammatory cytokines, such as IL-10, TGF-β and IL-35. Tregs suspend rather than promote the action of local immune cells in a contact-mediated manner. Suppressive function of Tregs is performed by various different mechanisms. First of all, as mentioned above, Tregs produce suppressive cytokines, such as IL-10 and TGF-β. In addition to this, high affinity IL-2 receptor is expressed by Tregs, so Tregs can bind to T cell growth factor IL-2 from cell environment blocking up the advance of local inflammation. Futhermore, Tregs may promote cell lysis via perforin and granzymes A and B pathways. Alteration of the function and maturation of dendritic cells is also mediated from Tregs for immune homeostasis. In addition to previous procedures, many other mechanisms have also been described in international bibliography. [3]

As far as it concerns patients with Inflammatory bowel disease (IBD), although it is already known that a downregulation of the immune system exists, the participation and role of human intestinal $Foxp3^+$ Tregs in this immune suppression are not completely understood. In a few studies, high presence of $Foxp3^+$ cells is noted in the lamina propria of the involved parts of the colon of IBD patients. [4], [5] In addition, the population of $Foxp3^+$ T cells in intestinal biopsies from patients with either Crohn's disease or ulcerative colitis is highly associated with the histological grade of local inflammation. [4]In order to explore Tregs inhibitory activity, it is mentioned that in patients with IBD, Tregs from the bowel are equally suppressive as those from healthy controls. [6], [7]

Because of the factors mentioned above, which are still quite obscure, the human immune system fails to fully control inflammation in the case of IBDs. How the presence of an infectious factor could influence immune response in similar patients with IBD? We attempted to study whether coexistence of CMV in patients colon promote or restrict Tregs from mediating tissue immune defense.

Cytomegalovirus is a member of the family of Herpesviridae, a double-stranded DNA virus that affects from 40% up to nearly 100% of general population. This is possible to happen either as a result of acute infection due to CMV or after

reactivation of a preceding infection. CMV infects immunodeficient patients much more often than immunocompetent patients. In the life cycle of CMV, the virus inducts a latent infection to macrophages and cells of the bone marrow mainly and to some other tissues. The fusion of infected cells leads to the formation of multinucleated giant cells and hence the virus was named Cytomegalovirus. [8] In 1961, CMV was associated with IBD for the first time when the virus was detected in a patient with ulcerative colitis. Since then, additional studies proved that CMV infection may constitute an activating factor in recrudescence of IBD. Therefore, CMV is accused of causing immunosuppression or aggravation of the symptoms of IBD. In most cases, CMV infection was a reactivation of a latent virus due to immunosuppressive medication that patients, while acute infections were rare. [9] According to bibliographic data, Tregs function after CMV infection remains immunosuppressive in an attempt to suppress local tissue inflammation. [3]

The present study aims to investigate T-regulatory cells'presence and role in samples of intestinal tissue from patients with inflammatory bowel disease when an infectious factor, such as CMV, further complicates the already existent local inflammation. We applied immunohistochemical methods (polyclonal antibodies against forkhead box protein 3-Foxp3-which claim to be the basic transcriptional factor for T regulatory cells) in 61 bowel tissue samples with inflammatory bowel disease, thirty of which concern patients with IBD complicated by CMV infection. Research results were aimed to be combined with data from the already existent relevant bibliography in an attempt to investigate Tregs role in immunodeficient patients with or without CMV infection.

Materials and Methods

A retrospective analysis of 61 formalin fixed, paraffin-embedded samples obtained from intestinal biopsies (mainly colonic) during diagnostic endoscopic examination or follow-up examination of already diagnosed patients derived from 61 patients bearing the diagnosis of Inflammatory bowel disease (either ulcerative colitis or Crohn's disease) was performed. In 31 of the collected cases no infectious factor had been detected, while in the rest 30 cases CMV's presence was detected. From every case paraffin sections with 4μm thickness were obtained. Hematoxylin-Eosin stained slides as well as immunohistochemically stained slides with CMV antibodies were evaluated. The samples were collected in a period of five years (2012-2016) in the Department of Pathology at General Hospital of Athens "G.Gennimatas" as well as the 1st Department of Pathology, Medical School, National and Kapodistrian University of Athens.

For immunostaining, the polyclonal rabbit antibody Tinto Foxp3 (supplied by Bio SB, USA) was used. It was diluted in buffer containing BSA and sodium azide as a preservative at pH 7.5. Serial sections of 4μm thickness were cut and subjected to 56^{o}C. Afterwards, deparaffinization, hydration and antigenic position opening was performed at pH 9.0. After heating and washing with buffer, the slides were incubated with primary antibody and in the next stage with the secondary antibody binded to peroxidase. After washing with TBST, chromogen DAB was infused and hematoxylin was used as a counterstain just before xylene and alcohol were added.

In order to estimate the level of expression, the number of Foxp3+ cells was measured in ten optical fields per slide and hence an average score was calculated for every sample. The results were statistically analysed in order to

correlate Tregs levels with CMV presence, disease activity and the quantity of eosinophils.

Statistical analysis was performed with R3.5.0. Statistical significance was set at 5% (α=0,05). The relation between the mean value of positive cells per optical field and other parameters were examined with Student's independent sample t-test. The relation between CMV presence, IBD activity and eosinophils were examined with Pearson's x^2 test.

Results

The study included 61 cases of inflammatory bowel disease (ulcerative colitis or Crohn's disease). In 30 of them CMV coexisted with IBD, while in the rest 31 cases no infectious factor was detected. As mentioned above, tissue samples were obtained from intestinal biopsies (mainly colonic) during diagnostic endoscopic examination or follow-up examination of already diagnosed patients and were studied immunohistochemically (polyclonal antibodies against Foxp3) in an effort to evaluate the quantity of regulatory T cells at the examined tissues.

Regarding IBD activation in patients, in 41 of the samples, signs of active inflammatory bowel disease were detected, whereas in the rest 20 cases the findings were suggestive of disease remission. Increased numbers of eosinophils were present in 42 patients, while in 19 cases no remarkable increase of the number of eosinophils was noticed. Tissue sample characteristics are listed in Table 1.

Statistical analysis reached the result that Foxp3 expression and, as a consequence, the number of regulatory T cells correlates with CMV presence in the examined samples. More specifically, Tregs were detected inlarger numbers in patient tissues where IBD coexists with CMV (p=0,041). On average, 85,25 regulatory T cells per ten optical fields were counted in tissues with IBD and CMV versus 59,86 Tregs in tissues from patients only with IBD. (**Figure 1**: slide with high expression of Foxp3 and increased number of Tregs, **Figure 2**: low Foxp3 expression and lower Treg number)

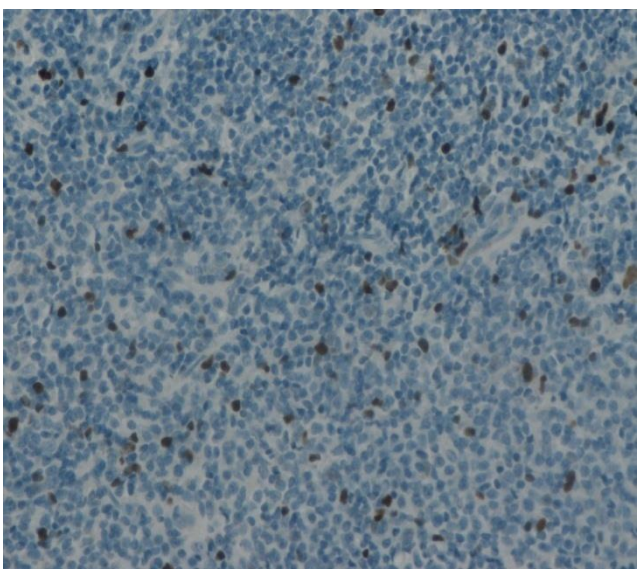

Figure 1.*High expression of Foxp3and increased number of Tregs(Immunohistochemistry, x200)*

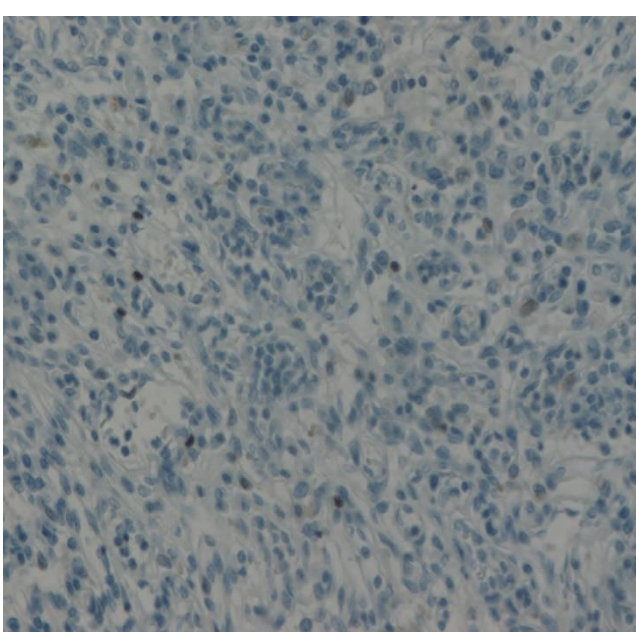

Figure 2. Low Foxp3 expression and decreased Treg number(Immunohistochemistry, x200)

The effect of other parameters, such as IBD activity and eosinophilia, on the Tregs number was also studied. Increased Tregs numbers were detected in patients with histological findings of active IBD. In addition, tissue samples with eosinophilia demonstrated high Tregs numbers, in contrast to those that were not characterised by an abundance of eosiphils.

Table 1. *CMV presence and tissue samples characteristics*

Characteristics		Immunohistochemistry cases		
CMV presence		CMV present	No CMV detection	Total
		30 (49,2%)	31 (50,8%)	61 (100,0%)
IBD activation	Active IBD	21 (51,2%)	20 (48,8%)	41 (100,0%)
	Inactive IBD	9 (45,0%)	11 (55,0%)	20 (100,0%)
	Total	30 (49,2%)	31 (50,8%)	61 (100,0%)
Eosinophilia	Increased number of eosinophils	15 (35,7%)	27 (64,3%)	42 (100,0%)
	Not increased eosinophils number	15 (78,9%)	4 (21,1%)	19 (100,0%)
	Total	30 (49,2%)	31 (50,8%)	61 (100,0%)

Results processed with statistical analysis disclosing correlation of evaluated parameters are collected in Table 2 with P values performance.

Table 2*. Statistical analysis of factors associating with Foxp3 expression*

Factors	P value (Foxp3 expression in colon biopsies)
CMV	0.041
IBD activity	0.014
Ssdd Eosinophilia	0.037

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CMV	0.041
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Discussion

In the majority of cases, cytomegalovirus infection causes no symptoms at all. After primary infection, CMV usually remains lifelong in a latent state. However, immunocompromised patients, such as patients with Crohn's disease or ulcerative colitis,are in a vulnerable position in regards to cytomegalovirus infection, in contrast to immunocompetent individuals. Patients with a past silent CMV infection may show symptoms of activation of this infection after getting immunocompromised due to an IBD or its treatment. [10]More recent experimental studies in murines also revealed a triggering role of CMV in the development of Crohn's disease and ulcerative colitis. [11]

It has been suggested that Inflammatory bowel diseases may practically be the clinical expression of diminished immunotolerance to commensal bacteria. Treg cells are critical for the immune tolerance in the intestine. Many studies have so far indicated that Tregs play a protective role in IBD. [12] Treg phenotype shows no alterations in patients with IBD despite the mucosal immune dysregulation. If wanted to characterize Treg phenotype concerning activation and inhibitory molecule, it may be characterized as a fully regulatory phenotype. [13]

Experimental data have revealed that T cell immune reactivity against antigens can be controlled by immunoregulatory T cells. However, if requested to examine these cells and functions, a specific marker for Treg identification is required. Recent researches concerning Foxp3 protein revealed that it can be used for the quantification and enumeration of T cells with regulatory potency. [14]

In our research, immunohistochemical staining with a polyclonal antibody against Foxp3 was used in order to identify Tregs. With the method described above we were enabled to evaluate Treg population at the colon tissue and compare its presence in IBD patients with and without CMV colon infection.

The results of our study showed that Tregs were detected in larger numbers in the samples from patients with IBD that coexisted with CMV. The number of Foxp3+ Tregs was significantly higher in the mucosa of IBD patients with CMV infection in comparison to IBD patients without any intestinal infection. This finding suggests a remarkable increase of Treg cell pool in the infected colon mucosa due to causes that remain to be further investigated and clarified.

It is of great interest the fact that according to several articles increased Treg numbers are present in CMV colitis and similar mucosal infections. Recent studies suggest that such infiltrates can be the outcome of local inflammation caused by viral or bacterial infections. [14] Immunohistochemical staining for

nuclear Foxp3 gives the advantage to identify and enumerate even very low Treg numbers. Given the fact that Tregs maintain regulatory phenotype in IBD patients, a suppressive activity on behalf of Tregs is expected in the inflamed mucosa accordingly to the grade of inflammation. [4] In similar investigation patterns many studies demonstrated that the depletion of Foxp3+ T cells in local mucosal tissues launched the development of GvHD-like reactions in target organs. [15], [16]

Further results with regard to Foxp3+ T cells and their correlation with IBD activity grade may be easily explained considering that in IBD the immune system makes an effort to transcend the slight immune deficiency that led to IBD outbreak and to get protected against it. [13] As a result, elevated numbers of Tregs are found in the intestinal mucosa and circulation of patients with IBD, when compared with healthy controls. [17]

Eosinophilia is generally considered a sign of chronic inflammation. In our research, increased presence of eosinophils was associated with higher Treg numbers. Inflammatory bowel diseases are undoubtedly medical conditions with a long clinical course. Therefore, a continuous chronic localinflammation is expected to derange mucosal Treg number. Some studies correlate chronic inflammatory diseases with Treg deficiency in peripheral blood. [14] However, Tregs quantity in peripheral blood does not reflect accurately their presence in target tissue. The mucosal sequestration of Tregs may offer a presumable explanation of their paucity in blood circulation. [3], [14], [18], [19]

Conclusion

In conclusion, the findings of our study demonstrate that Treg infiltration in inflamed mucosal tissues of IBD patients show a significant increase when CMV infection coexists in comparison with IBD patients without any intestinal infectious disease. Treg cells play a crucial role in suppressing local inflammation induced by viral intestinal infections and their regulatory potential may improve the state of intestinal mucosa by providing an active defensive mechanism against CMV, even in patients with partial immune deficiency, like IBD patients. The influence of Tregs over viral colon infection of patients with IBD should be further studied retrospectively and prospectively in order to determine whether these Foxp3+ T cells can be of value in a therapeutic context in order to control ongoing inflammation

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Diode type laser in resection biopsies of superficial benign lesions of oral mucosa: a histological analysis

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Abstract

Aim: The aim of this study is to assess whether the thermal damage caused by the diode laser during the excision of benign lesions of the oral mucosa affects the histopathological diagnosis or the safe evaluation of fibroepithelial lesions' excision margins. In addition, a comparison of the surgical margins' histological appearance between benign fibroepithelial lesions of the oral mucosa subsequent to diode laser treatment and traditional surgery is attempted.

Materials and Methods: Out of 60 cases, 30 were treated surgically and 30 with diode laser. All lesions were benign and excised on clear margins using either method (surgical or laser). The removed specimens were examined using a light microscope aided by an image analysis method, while the thickness of thermal necrosis zone was also measured.

Results: This analysis concluded that the thickness of the thermal necrosis zone, measured by the image analysis method in our material, is directly proportional to the type and the dimensions of the lesion, suggesting that the laser method of excision is an alternative method potentially characterised by selectivity and accuracy, with regard to the removal of tissue lesions. Laser penetration depth during the resection of the lesions can be controlled. The hemostatic property of laser was apparent as well as the reduction of fibrous connective tissue, leading to faster recovery.

Conclusion: Image analysis method illustrated that the thickness of the thermal necrosis zone permits clear histological margins that guarantee the clean and complete excision of lesions. Based on the above, expanding the use of laser in the excision of malignant lesions of the oral cavity should definitely be considered.

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Introduction

Although, in dentistry, laser has been used in conservative therapeutic treatment of oral disorders such as periodontitis and peri-implantitis, its use in oral surgery still remains in a primary stage [1-4].

Over the past few years, a large number of clinical, laboratory and research data has been collected on the application of different wavelength laser techniques in the excision of benign lesions of the oral cavity [5-9]. The use of laser in surgical treatment offers advantages to both surgeons and patients. The rapid and accurate handling of surgical techniques improves therapeutic remedial restoration and alleviation of post-operative symptoms (such as pain, edema, hyperemia) [10-11].

The most common lesions of the oral cavity (benign tumors, chronic papillary inflammatory processes, cysts, etc.) are structured by the covering multi-layered squamous epithelium and the underlying subepithelial connective tissue of the oral mucosa, and resemble similar lesions to other anatomic locations.

Hyperplastic fibroepithelial lesions are the most frequent tumor-like processes associated with chronic injuries (buccal, lip and tongue biting). They are painless masses of fibrous connective tissue, which are stable for years, while they are covered by smooth and pinkish mucosa without ulceration [12]. The histological features of benign lesions consist of hyperplastic squamous epithelium, often in the form of pseudo-epitheliomatous hyperplasia, while the subepithelial connective tissue is generally cellular, composed of widely dispersed mature fibroblasts. Chronic perivascular inflammatory infiltrate is commonly identified, especially when the concurrent presence of Candida albicans superinfection is appreciated, or the existence of precancerous dysplastic epithelial changes is observed. The diode laser has been reported extensively as contributing significantly to the treatment of benign vascular and precancerous lesions of the oral mucosa [13-16].

The accuracy and clarity of pathological diagnosis and the extent of thermal damage due to laser are contradictory points for the widespread use of the surgical laser method. Nonetheless, careful invasive manipulations are expected to significantly reduce the thermal damage of the laser and improve the process of tissue healing [17-19].

The purpose of this study is to assess whether the thermal damage caused by the diode laser affects the histopathological diagnosis or the evaluation of benign lesions' excision margins. In addition, a comparison between the histological appearance of benign fibroepithelial lesions of the oral mucosa after surgery with laser and conventional surgical treatment is attempted.

Materials and Methods

Our sample consisted of 60 cases of benign lesions of the oral cavity mucosa.

A 984nm diode type laser was used in half of the cases. Out of 60 respective patients, 41 were women aged 14 to 82 years old and 19 were men aged 10 to 63 years old.

The material consisted of cases that corresponded in order of frequency to reactive fibroma (29 cases), inflammatory papillary hyperplasia (9 cases), papilloma (6 cases), epulis (3 cases), leukoplakia (3 cases), mucus cysts (3 cases), pyogenic granulomas (2 cases), angiokeratomas (2 cases), epidermal cysts (2 cases) and fibroepithelial polyp (1 case).

The size of the lesions ranged from 3 mm to 15 mm. The most common locations the lesions were gingiva, tongue, and mucous membrane of lips, cheeks, tongue, and hard palate.

A diode laser type with a wavelength of 980 nm, a penetration depth of 3-5 mm and energy of 2-3 watt was chosen for laser treatment. A pulse wave was used in order to avoid thermal damage. Out of 60 cases in total, 30 were treated surgically and 30 with diode laser.

More specifically, 15 reactive fibromas, 5 inflammatory papillary hyperplasias, 3 papillomas, 2 leukoplakias, 2 mucus cysts, 2 epulis and 1 angiokeratoma were treated with laser, whereas 14 cases of traumatic fibroma, 4 cases of inflammatory papillary process, 3 cases of papilloma, 2 cases of pyogenic granuloma, 2 cases of epidermal cyst, 1 case of epulis, 1 case of leukoplakia, 1 case of mucous cyst, 1 case of angiokeratoma and 1 case of fibroepithelial polyp were removed surgically (Fig. 1).

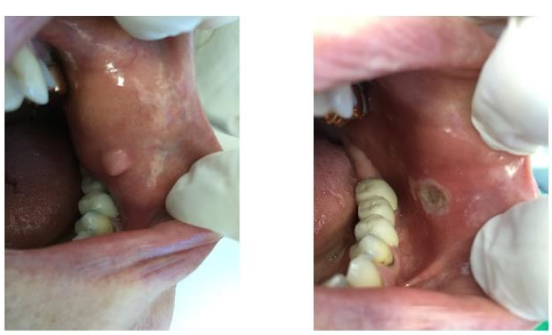

Figure 1. *Fibriepithelial polyp of buccal mucosa: a. preoperatively, b. after laser excision*

In most cases, only local anesthesia was administered using a cotton dipped in lidocaine. The patients were re-examined 3 days, 1 week and 3 weeks after the intervention.

Following the excision, the tissue was fixed in 10% neutral formalin solution for 2 days and embedded in paraffin. Using a microtome, 3mm thick histological sections were taken and stained with the Eosin-Hematoxylin method. Microscopic evaluation was performed under a light microscope using image analysis. The thickness of the thermal necrosis zone was measured on the lesions removed with diode laser and the thickness of the healthy tissue which was excised with the scalpel in the cases conventionally treated.

Results

Microscopically, all lesions were nonmalignant and removed on healthy margins regardless of the applied method (conventional surgery or laser).

In surgically excised tissues, the thickness of healthy tissue excised peripherally to the lesion was around 1mm in most cases. However using the laser method, the thermal necrosis zone formed in the lesion's background and adjacent tissues, can offer distinct and clear excision margins (Fig. 2).

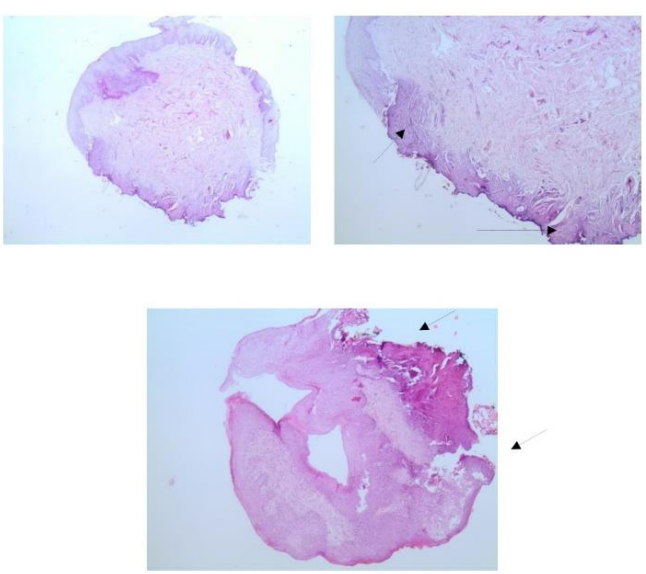

Figure 2*. Histological images of traumatic fibroma (up) and mucocele (down). Note the thermal necrosis zone (arrows)*

The thickness of the thermal necrosis zone, measured by the image analysis method in our material, ranged from 101 to 192 μm. Table 1 shows that this thickness is directly proportional to the type and size of the lesion. Large-diameter lesions with large amount of fibrous connective tissue have a thicker thermal necrosis, whereas small-diameter lesions with less fibrous connective tissue have a thinner one. These findings implicate that laser penetration depth during the resection of the lesions can be controlled when the type and size of the lesions are considered and the parameters of the laser applied per case are controlled, based on the laser method selectivity and accuracy.

In laser excised tissue, a significant reduction in scar tissue and tissue shrinkage was observed, thus leading to faster recovery.

Finally, it is worth noting that in all cases, the hemostatic property of the laser was apparent, giving a visible surgical field. It seems that the thermal necrosis zone caused by the laser, acts as a hemostatic plug.

Discussion

In the studied cases, we observed that laser interacts differently with different type of tissues. This is justified by the different penetration rate of the laser, which depends on the composition and size of the tissue, as well as on the parameters of laser such as wavelength, pulses, Hertz, and Joules. More specifically, different types of tissues absorbed different amounts of laser energy. The oral tissue is occasionally pigmented; melanin, hemoglobin and collagen chromophores, due to the chromophilic property of the diode laser, absorb the laser energy better. In addition, the tan oral mucosa can absorb the laser's energy quickly and thus give the surgeon a quick and precise cut [11-13].

Furthermore, the removal of edematous and hyperemic tissue was clearly more rapid as opposed to relatively healthy tissues. This was due to the high content of the former tissues in dilated capillary vessels and increased amount of interstitial liquid, which facilitated the absorption of laser energy [11-13].

The tissue thickness affected the effectiveness of the diode laser. In thin mucous membranes with a small amount of fibrous connective tissue, such as in mucocele excisions, the laser has been shown to be a particularly effective and fast tool (1-2 watts), as opposed to some traumatic fibrous connective tissues, in which the large amount of connective tissue absorbed the laser energy, and made it necessary to increase it up to 3 watts).

It is a fact that in laser treated specimens tissue clotting is secondary. No bacterial contamination was observed due to the bactericidal property of the laser and the protective layer of fibrin created directly, which give the tissue the possibility of rapid healing.

The non-injecting nature of anesthetic surgery, as well as the precise cutting of the laser, ensured minimal mechanical injury and offered reduced postoperative pain and edema.

It should be pointed out that the laser-induced thermal damage has in no way affected the histopathological diagnostic procedure of the lesions in all cases. The advantages in using the diode laser method include: reduced pain, less visits to the doctor, postoperative progression, non-suture of the wound, hemostasis and avoidance of unnecessary anesthesia. The disadvantages are the risk of thermal damage to the underlying and adjacent tissues in the application area, in case of malpractice, as well as the increased cost [11-13,17]

Comparing the excision of lesions with diode laser and scalpel, we concluded that for intraoral soft tissue surgical techniques, laser is a reasonable alternate to the scalpel [20].

Conclusions

Our analysis, using the application of image analysis method, concluded that the thickness of the thermal necrosis zone permits clear histological margins that guarantee a safe and complete excision of the lesions, while also controlling the penetration degree of the laser during the tissues' cutting. It should be taken into account that similar thermal necrosis thickness is also present in the surgical field of the tissue after the lesion is removed which, ultimately, extends the clear margins of excision.

Based on the above, expanding the use of laser in the excision of malignant lesions of the oral cavity should definitely be considered. In oral mucosal lesions such as leukoplakia (focal or multifocal), high-grade epithelial dysplasia, in situ carcinoma and microinvasive carcinoma, the use of the laser can provide positive results. This was also evident in the cases of leukoplakia of our material. Moreover, laser surgery has already been applied to the excision of dysplastic lesions, in situ carcinomas and microinvasive carcinomas of the breast, urinary bladder and gastroesophageal junction.

It should be emphasized that both methods (laser and conventional surgery) beyond any of the advantages or disadvantages described above, can provide the patient with safe and complete therapeutic effects..

Table 1. Thermal necrosis zone thickness of the excision sites removed with laser, measured with image analysis method

Lesion	Lesion Diameter (mm)	Thermal necrosis zone thickness (μm)
1. Lower lip fibroma	3	121.15
2. Buccal fibroma	4	139.62
3. Hard palate fibroma	14	190.39
4. Buccal fibroma	6	142.13
5. Lower lip fibroma	5	144.48
6. Lower lip fibroma	3.5	121.54
7. Tongue fibroma	6.5	152.25
8. Frenulum	3.5	126.65

9. Tongue fibroma	8	169.22
10. Lower lip fibroma	4	127
11. Tongue fibroma	12	190.44
12. Buccal fibroma	6.5	142.35
13. Buccal fibroma	7	149.24
14. Tongue fibroma	5	139.45
15. Buccal fibroma	15	192
16. Papillary inflammatory gingival hyperplasia	5	106
17. Papillary inflammatory gingival hyperplasia	8	141.22
18. Papillary inflammatory gingival hyperplasia	4	103
19. Papillary inflammatory gingival hyperplasia	5	152
20. Papillary inflammatory gingival hyperplasia	3.5	124
21. Upper lip fibroma	3.5	124.26
22. Lower lip fibroma	4	105
23. Tongue fibroma	3.5	129
24. Lower lip leukoplakia	3	112
25. Lower lip leukoplakia	3	131.77
26. Epulis	6	121.82
27. Epulis	6	139.62
28. Lower lip mucocele	3	136.28
29. Lower lip mucocele	3	102
30. Upper lip angiokeratoma	3	101

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Immunohistochemical Investigation of β-catenin Expression in Renal Cell Carcinoma

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Abstract

Renal cell carcinoma is the most common renal malignancy and accounts for approximately 5% of all cancer diagnoses in adults. One of the molecular mechanisms that have been implicated to play an important role in renal cell carcinogenesis is the disregulation of Wnt signaling pathway, reflected by the abnormal expression of beta-catenin. For the purpose of our study, we selected twenty (20) cases of renal cell carcinomas of clear cell, papillary and chromophobe types, confirmed through pathological reevaluation, in order to assess the expression levels of β-catenin. Immunohistochemical staining with anti-β-catenin specific monoclonal antibody was performed. Our study provides hints of the involvement of β-catenin in renal cell oncogenesis.

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Introduction

Renal cell carcinoma is the most common renal malignancy and accounts for approximately 5% of all cancer diagnoses in adults. Renal cell cancer has proven to be a rising cause of morbidity and mortality during the last few decades, making more and more demanding the need for discovering its molecular background and the potential therapeutic interventions. Moreover, apart from the surgical approach based on radical or partial nephrectomy, very few drugs have been so far approved as treatment options, when dealing with a patient with renal cell carcinoma [1].

Until recently, the underlying molecular mechanisms of renal cell oncogenesis were quite unclear. However, remarkable research has been conducted in this field during the last few years, leading to the identification of various disregulated molecular pathways in the pathogenesis of renal cell carcinoma, such as PI3K/Akt/mTOR, HGF/c-Met and MAPK. One of the molecular mechanisms that has been implicated to play an important role in renal cell carcinogenesis is the disregulation of Wnt signaling pathway, reflected by the abnormal expression of beta-catenin [2].

Beta-catenin is a member of the catenin protein family involved in the regulation and coordination of cell-to-cell adhesion and gene transcription. Signal transduction pathways implicating β-catenin have proven to be involved, as common mechanisms, in the carcinogenesis of various organs [3].

For the purpose of our study, twenty (20) cases of renal cell carcinoma of clear cell, papillary and chromophobe types, confirmed through pathological reevaluation, were selected. In order to evaluate the expression levels of β-catenin an immunohistochemical evaluation using an anti-β-catenin specific monoclonal antibody (mouse monoclonal, clone 14, 1:200 dilution, Bio SB, Santa Barbara, CA, USA) was performed. The peritumoral renal cell tissue was used as a normal control for the immunohistochemical assessment.

The immunohistochemical evaluation showed that in all cases of renal cell carcinoma, the expression level of β-catenin was increased compared with normal peritumoral tissues. More specifically, the entire group of renal cell carcinomas, regardless of histopathological subtype, showed strong membranous and/or cytoplasmic staining patterns (Fig. 1)

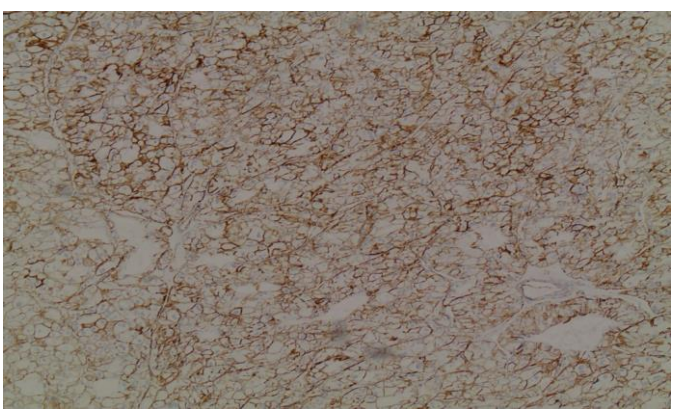

Figure 1*. Membranous immunohistochemical staining for β-catenin on formalin-fixed, paraffin-embedded section from a case of clear cell renal cell carcinoma (original magnification x200)*

During the last few decades several studies have been performed in order to elucidate the structure and functions of β-catenin. However, the exact role and mechanism of action of β-catenin in renal cell carcinoma remains unclear [4]. Our study provides hints of the involvement of β-catenin in renal cell oncogenesis.

In conclusion, further research is needed in order to evaluate the variable expression of β-catenin in renal cell cancer, the importance of the immunostaining expression pattern - membranous and cytoplasmic in contrast to nuclear -and the potential use in clinical therapeutics.

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EBV-positive mucocutaneous ulcer affecting the bowel

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Abstract

Epstein-Barr virus (EBV)-positive mucocutaneous ulcer (EBVMCU) was included in the latest edition of the WHO classification of tumors of hematopoietic and lymphoid tissues as a distinct clinicopathological entity. The disease is characterized by well demarcated indurated ulcers occurring in cutaneous and mucosal sites including the gastrointestinal tract. Herein, we report two new cases of this peculiar disease. The first case presents as large bowel obstruction mimicking malignancy, the second as acute abdomen due to small bowel perforation. Histology discloses characteristic B cell blasts with Hodgkin/Reed-Sternberg cell like-features, which are positive for EBV on EBER in situ-hybridization. Clinical implications and differential diagnosis, including distinction from classical Hodgkin lymphoma and lymphomatoid granulomatosis are discussed.

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Introduction

Epstein-Barr virus (EBV)-positive mucocutaneous ulcer (EBVMCU) was proposed by Dojcinov et al. in 2010 as a distinct clinicopathological entity [1]. Lesions may be found in oropharyngeal mucosa, skin, and gastrointestinal tract [1, 2].

Since the original description, approximately 100 cases have been described in the literature [3], approximately 20 of them within the gastrointestinal tract [4]. In general, affected individuals are immunocompromised, due to iatrogenic immunosuppression or age-related immunosenescence [1, 5].

On gross inspection, the disease presents as well demarcated indurated ulcer. The histology is characterized by a polymorphous cell infiltrate, containing large B cell blasts with Hodgkin/Reed-Sternberg (HRS) cell like-features, which suggests classical Hodgkin Lymphoma (cHL) as main differential diagnosis [1, 2, 5].

Herein, we report two new cases of EBVMCU that occurred within the gastrointestinal tract of two individuals not receiving immunosuppressive medication. We describe the morphological characteristics of the disease and discuss the differential diagnosis.

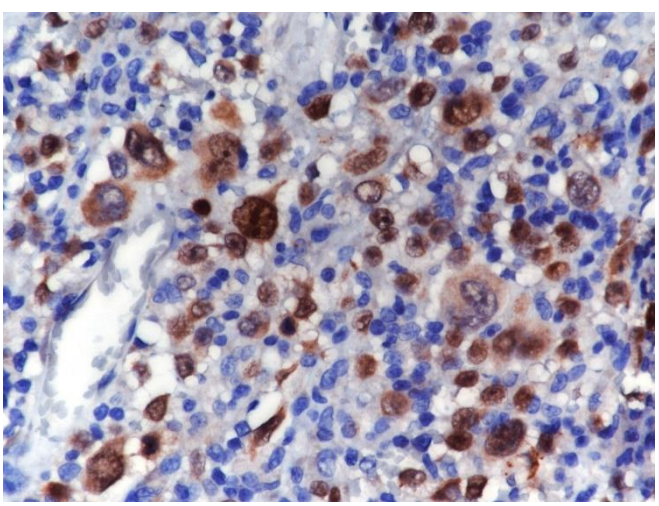
Case 1

A 54-year-old female presented with unspecific abdominal pain, obstipation and iron deficiency anemia. Endoscopy revealed stenosis of the sigmoid colon that was suspicious for malignancy, prompting left-sided hemicolectomy. The resection specimen contained an ulcerated mass lesion, three centimeters in maximum diameter.

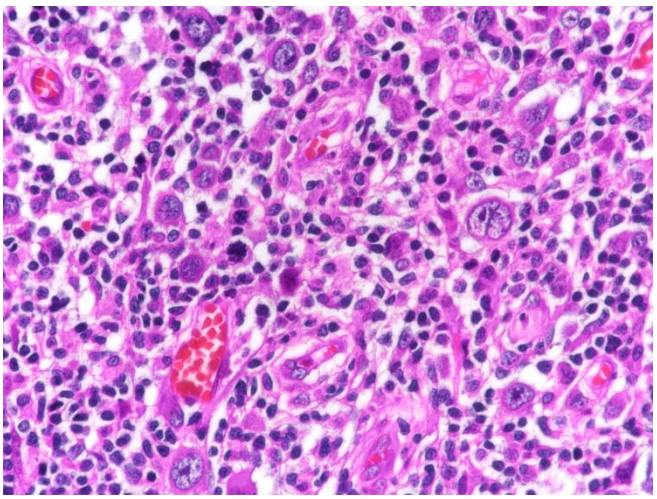
Histology showed a diffuse infiltrate of large cells with immunoblastic morphology, but blasts with HRS cell like-features, including binucleated cells, were also present (Figure 1A). Mitotic figures were frequently observed. Lymphocytes prevailed in the background, admixed with plasma cells and occasional granulocytes. The resected lymph nodes were not involved.

Immunohistochemistry proved the blasts to be diffusely positive for PAX5, CD15, and CD30 (Figure 1B-D), while only occasional cells were positive for CD20. The MIB-1 proliferation rate was 70% (Figure 1E). The infiltrate was strongly positive for EBV applying EBV encoded small RNAs (EBER) *in situ*-hybridization (Figure 1F).

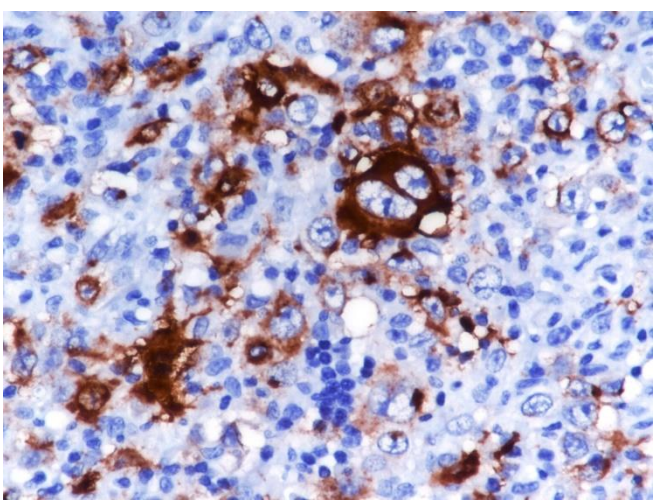
Figure 1: *The large bowel ulcer contains a diffuse infiltrate of large cells with mainly immunoblastic morphology, but also blasts with HRS cell like-features, including binucleated cells, against a background of mainly lymphocytes and rare plasma cells*



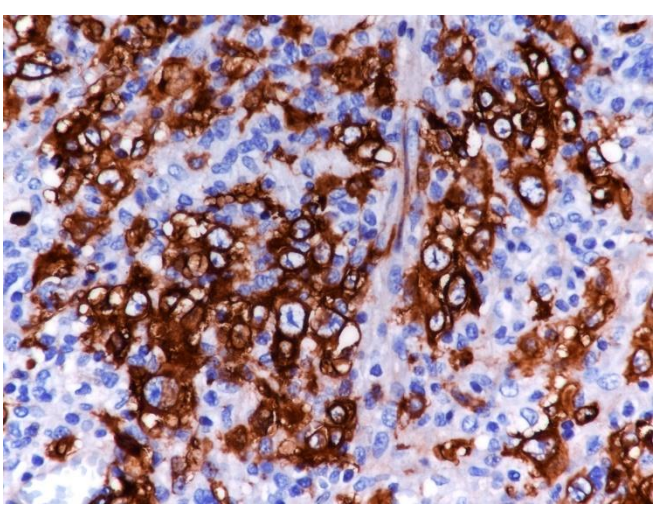
A; *original magnification x400). Immunohistochemistry proves the blasts to be diffusely positive for PAX*



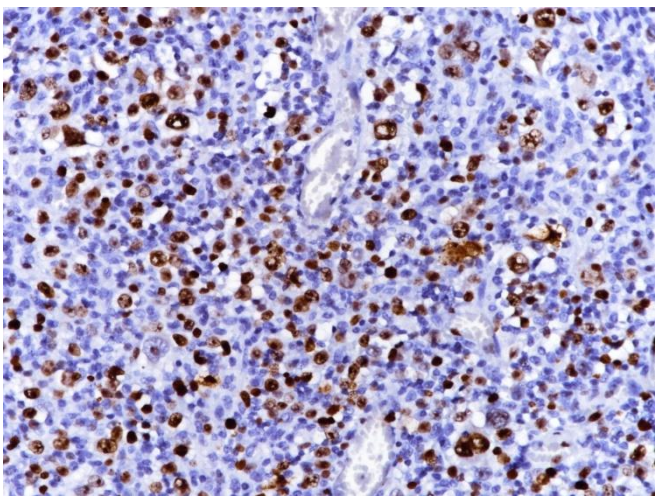
B; original magnification x400), CD1



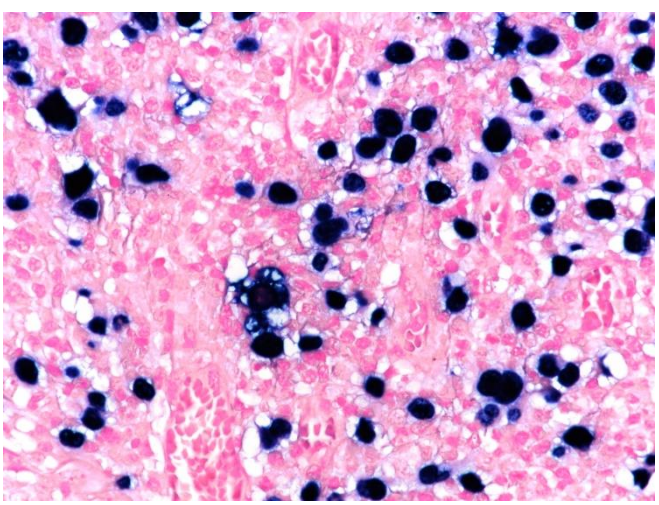
***C;** original magnification x400), and CD30*



***D;** original magnification x400). The MIB-1 proliferation rate is 70%*



***E;** original magnification x400). The blasts are strongly positive for EBV applying EBV encoded small RNAs (EBER) in situ-hybridization*



***F;** original magnification x400).*

Case 2

A 75-year-old female presented with acute severe abdominal pain. CT scan disclosed perforation of the small bowel and segmental resection was performed. The operation specimen demonstrated a deep penetrating ulcer with thickening of the bowel wall and frank perforation.

Upon histology, prominent granulation tissue with mixed cellular infiltrate rich in lymphocytes and plasma cells was identified, extending beyond the bowel wall into the mesenteric adipose tissue. The infiltrate contained scattered polymorphous blasts with HRS cell like-features and high mitotic count. The resected lymph nodes were not involved.

Immunohistochemistry was performed, rendering the blasts positive for CD15, CD20, and PAX5. As in the previous case, EBER *in situ*-hybridization was positive, particularly in the polymorphous blasts with HRS cell like-features.

Discussion

We presented two new cases of gastrointestinal EBVMCU that were diagnosed in patients not receiving immunosuppressive medication. Information regarding other causes of inborn or acquired immunosuppression, including HIV-status was not available. The first lesion mimicked a large bowel malignancy, in the second patient small bowel perforation caused acute abdomen.

EBVMCU represents a newly recognized clinicopathological entity that is characterized by sharply circumscribed indurated mucosal or cutaneous ulcers [1, 2]. The pathogenesis of disease has been related to altered immune function, hence EBVMCU is generally observed in patients under immunosuppressive therapy, e.g. after transplantation [6], in inflammatory bowel disease [1, 7, 8] or rheumatoid arthritis [1, 4, 9]. In patients not receiving immunosuppressive agents, age-related immunosenescence is believed to be the main causative factor [1, 5]. It is of note that the disease has rarely been observed also in HIV-positive individuals [10].

Prognosis is generally favorable, and even spontaneous regression has been reported [1, 5]. Due to the self-limited benign growth potential, conservative management is usually sufficient, and clinical remission can be achieved by modification and/or reduction of immunosuppressive therapy alone [5]. In cases of age-related immunosenescence or lack of response to change of immunosuppressive therapy regimen, additional radio- or chemotherapy may be necessary [1, 5, 10]. In our patients no adjuvant treatment was given after total surgical resection of the lesion and recurrence was not observed.

Two distinct histological patterns of EBVMCU have been described: The first pattern is characterized by a diffuse, predominantly “monomorphic” infiltrate of large cells, most of them with immunoblastic morphology. The second pattern shows a “polymorphic” infiltrate composed of some blast with HRS-like features, and dense accompanying infiltrate with medium-sized lymphocytes, plasma cells, and occasional neutrophils and eosinophils [6, 10].

Upon immunohistochemistry, EBVMCU demonstrates strong expression of CD20, CD30, CD15, and PAX5 in most cases. Crucial for diagnosis is however the proof of association with EBV infection. This can easily be achieved by EBER *in situ*-hybridization [2, 5, 10].

Differential diagnosis mainly includes classical Hodgkin Lymphoma (cHL), but also lymphomatoid granulomatosis (LYG) [2]. Within the gastrointestinal tract, cHL is exceedingly rare and it is conceivable that most cases of EBVMCU have been misdiagnosed as cHL in the past. Our case 1 may in fact serve as a good example since it had initially been labelled as cHL by the referring pathologist. LYG is an angiocentric and angiodestructive lymphoproliferative disease containing EBV-positive B cells, which in grade 3 disease may show atypia reminiscent of Hodgkin cells. The disease involves extranodal sites,

preferably the lungs, yet only rarely the gastrointestinal tract [11, 12]. Table 1 summarizes the morphological features of EBVMCU, cHL, and LYG, including immune profiles and EBV staining results.

In conclusion, EBVMCU is a rare disease that occurs in cutaneous and mucosal sites including the gastrointestinal tract. Pathologists need to be aware of this peculiar lesion, as misdiagnosis, particular as cHL, implies severe clinical implications for the affected individual with respect to potential overtreatment.

Table 1. Morphological features separating EBV-positive mucocutaneous ulcer (EBVMCU) from classical Hodgkin Lymphoma (cHL) and lymphomatoid granulomatosis (LYG)

	EBVMCU	**cHL**	**LYG**
Blasts with Hodgkin/Reed-Sternberg cell like-features	++	++	+/-
Bland-looking mixed inflammatory cell background rich in lymphocytes and plasma cells	++	++	++
Angiocentric and/or angiodestructive pattern	+/-	-	++
Necrosis	+/-	+/-	++
CD15	+	+	+/-
CD20	++	+/-	++
CD30	++	++	+
PAX5	++	+	Not available
MUM1	++	++	Not available
EBER *in situ*-hybridizaion	++	+/-	++

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Metastatic Urothelial Carcinomas with high serum level of Ca19-9. apropos of three cases

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Abstract

CA 19-9 immunoexpression in cytological material, as shown by few case reports in the literature, may have a diagnostic significance in urothelial carcinoma, especially in cases of metastatic disease with unknown primary.

In the present paper, we present three cases of metastatic urothelial carcinoma with elevated CA 19-9 serum levels. The lesions were located in the lung, ascitic fluid and the skin of the right inguinal region. Paracentesis was done and ThinPrep smears were prepared for cytological diagnostic purposes. For immunocytochemical investigation, antibodies against cytokeratins 7 and 20 and GATA binding protein 3 (GATA-3) were included as well as the monoclonal antibody against CA19-9.

Morphological and immunocytochemical examination (positivity for CK7, GATA-3 and/or CK20), revealed the urothelial origin in these three cases. ABC-peroxidase staining also showed the presence of CA 19-9 in some tumor cells.

Metastatic urothelial carcinoma may express CA19-9, as detected immunocytochemically in our cases, and may also cause elevation of CA19-9 serum levels.

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Introduction

Urothelial carcinoma (UC) usually does not present with metastatic disease, but when distant metastases occur, most commonly, they involve bone, lung and liver [1]. Metastatic UC can be early diagnosed with relative ease accuracy by combining the clinical presentation with the cytomorphologic appearance [2, 3]. In some instances, however, because of lack of tumor differentiation as well as of morphologic similarity among tumors of different origin, it may be difficult to recognize UC cells by routine stains. Today, for the most part, immunocytochemistry provides us with a useful tool in the diagnosis of neoplastic origin. The selection of appropriate immunocytological markers to aid in the diagnosis of an undifferentiated neoplasm depends on a number of factors including clinical findings, cytological appearance and differential diagnosis [4, 5]. ThinPrep processed smears are suitable for immunocytochemical studies. In general, some biomarkers have better sensitivity than does cytology alone. Although each single marker demonstrates low specificity, immunocytochemical markers have a potentially important role when used in combination [6, 7].

CA19-9 is a tumor marker which has been widely used for adenocarcinoma of the upper gastrointestinal tract, particularly primary adenocarcinoma of the pancreas; it also has been reported to be positive in gastrointestinal carcinomas, thyroid papillary carcinomas and endometrial adenocarcinomas [8]. In bladder cancer, CA19-9 has seldom been clinically used as a tumor marker. However, it has recently been reported that increased serum CA19-9 levels are associated with poor outcome in patients with urothelial carcinoma of the bladder [9, 10]. In the present report, we present three cases of metastatic UC with markedly elevated serum CA 19-9, including their immunocytochemical profile.

Presentation

Case (1)

A 74-year-old man admitted to the hospital with fever, cough and dyspnea of four days duration. Chest radiography and computerized tomography (CT) revealed lung lesions which were sampled by percutaneous fine needle aspiration (FNA) for cytological purposes. A Tru-cut needle biopsy was also taken for histological examination. The patient had previously been diagnosed with invasive UC, which had been treated with total cystectomy, ileal conduit diversion and urostomy, at the age of 70.

Case (2)

A 72-year-old man presented with abdominal swelling, lower abdominal pain, constipation and weight loss. Paracentesis of ascitic fluid was done and sent to the laboratory for cytological examination with the clinical indication that an intestinal primary was of a suspected intestinal primary carcinoma. Four years ago earlier, the patient was diagnosed with muscle- invasive bladder cancer (grade III and stage pT2N0M0), not eligible for cystectomy. Transurethral resection (TUR) was done and radiation therapy followed. The patient also had a history of resected colorectal adenomatous polyps.

Case (3)

An 80-year-old man presented with cutaneous swelling at the right inguinal region. The patient had a past history of radical cytectomy. Fine needle aspiration (FNA) was done.

All these cases were characterised by elevated CA 19-9 serum levels (> 100U/ml).

For morphological diagnosis, the aspirated cytological material was washed in CytoLyt solution (a methanol-based, buffered solution to lyse red blood cells) and placed into a processor (ThinPrep processor2000, Cytyc Corporation) for only one thin layer slide preparation from each

case; the ThinPrep slides were immersed into sodium phosphate buffer solution (pH 7.2) for 5 minutes, followed by the laboratory's standard Pap staining protocol. Immunocytochemical staining was done on smears prepared from the residual material of the ThinPrep fixed cellular sample. The panel of monoclonal antibodies used, included CK20, CK7, GATA-3 and CA19-9. Immunoperoxidase staining procedure was performed using the automated Dako Autostainer (Dako, Glostrup, Denmark) and diaminobenzidine was used as chromogen (ChenMate Envision Detection Kit Peroxidase/DAB, Rabbit/Mouse).

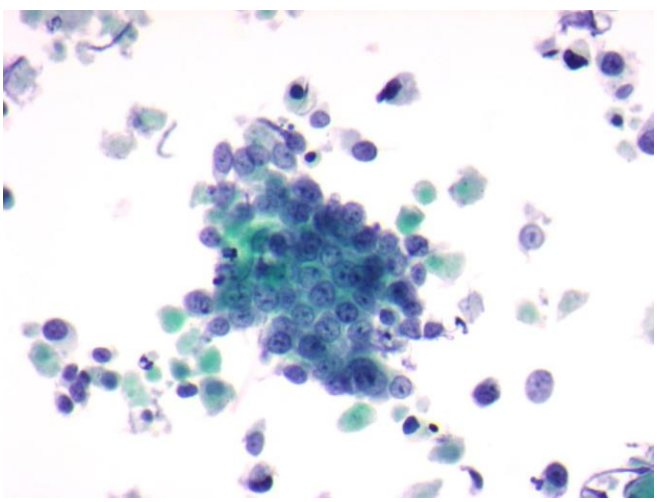

Figure 1. *Fine needle aspiration of a lung mass shows malignant cells in a characteristic two dimensional pattern (Papanicolaou x 200)*

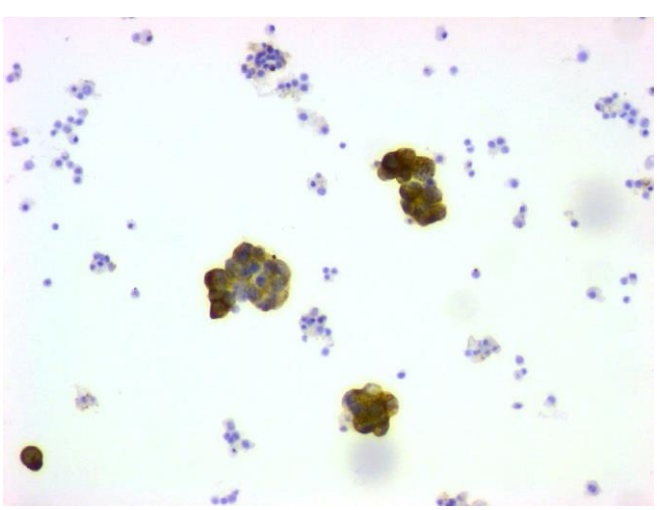

Figure 2. *Ascitic fluid involved by metastatic UCC shows diffuse membrane CK 7 reactivity (anti-CK 7 with 3,3 diaminobenzidine (DAB) chromogen)*

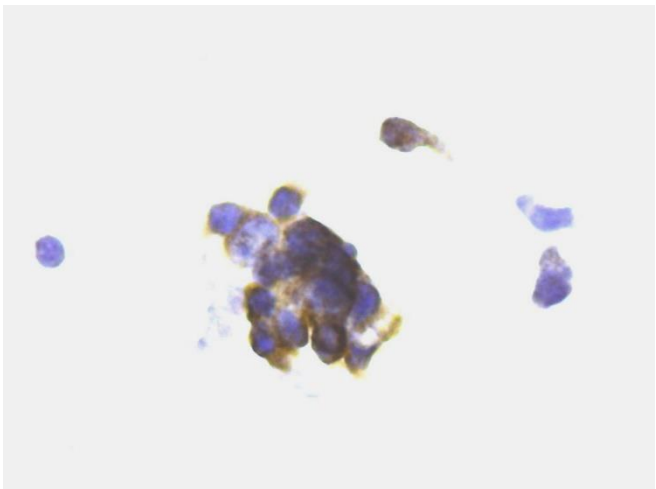

Figure 3. *Positive cytokeratin 20 immunoreactivity (immunoperoxidase method x 200)*

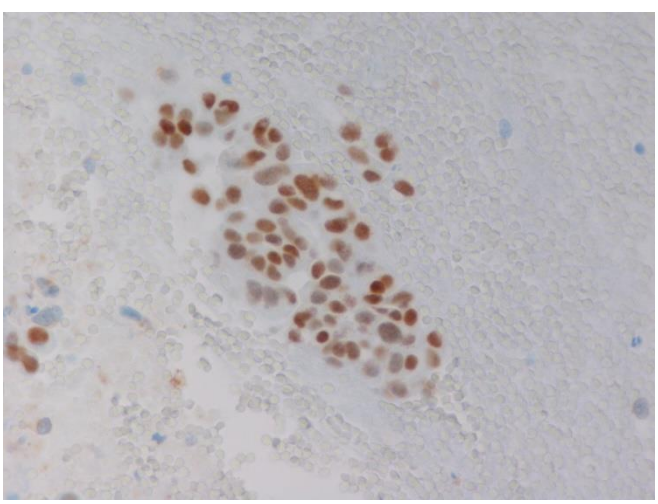

Figure 4. *Strong nuclear expression of GATA-3 (IMMUNOPEROXIDASE METHOD X 200)*

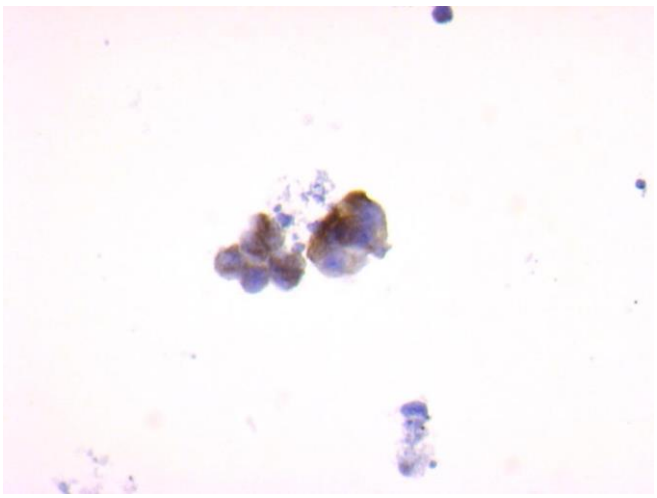

Figure 5. *Malignant cells with focal granular cytoplasm staining with CA19-9 antibody (immunoperoxidase x 200)*

Results

Cytological Finding

Morphologically, the first case was reported as a metastatic undifferentiated carcinoma. The second case was diagnosed as a metastatic carcinoma, probably of urothelial origin, while cytological diagnosis of the third case was metastatic urothelial carcinoma.

Immunocytochemical Results

All cases showed strong homogenous positive reaction to CK 7 and GATA-3, while the staining intensity against CK 20 was faint. Also, in all reported cases CA 19-9 immunopositivity was detected in a portion of the neoplastic population. Detailed immunocytochemical results are shown in Table 1.

Table 1. Antibodies contributed in the diagnosis of metastatic UCC

CASES/ MARKER	**CK 7**	**CK 20**	**GATA-3** (nuclear positivity)	**CA 19-9**
Case 1	+++ >90%of neoplastic cells	+ >60%of neoplastic cells	+++ >80% of neoplastic cells	++ in 20%of neoplastic cells
Case 2	++ >80%of neoplastic cells	-	+++ >80% of neoplastic cells	+ in 15% of neoplastic cells
Case 3	++ >90% of neoplastic cells	+ >50% of neoplastic cells	+++ >60% of neoplastic cells	++ in 25% of neoplastic cells

\+ : weak intensity, ++ : moderate intensity, +++: strong intensity

Discussion

A significant number of UCs of the bladder are primarily invasive or can subsequently progress to invasive and/or metastatic disease [11,12]. Flat tumors (carcinomas in situ, CIS) frequently progress to invasive disease [13]. Distant metastases occur largely in bone, lung and liver. Metastatic urothelial tumors may be difficult to distinguish from other poorly differentiated carcinomas because they are commonly positive for CEA [14]. Although a number of monoclonal antibodies directed against UCs have been produced in recent years, relatively few reports have been published on the definite use of such antibodies to diagnose UC. The study by G.P.Paner et al. [15] showed that GATA-3, S-100p, CK7, CK20, High Molecular Weight Cytokeratins and p63, in the appropriate differential diagnostic setting, may have important implications in the diagnosis of unusual bladder cancer histologies.

It is known that there are two different molecular pathways in UC pathogenesis:

a) papillary pathway that leads to hyperplasia and superficial papillary tumors and

b) non papillary pathway that leads to in situ lesions, invasive non papillary tumors and metastasis.

The utility of combined cytokeratin 7 and 20 immunoreactivity provides significant help in the identification of the primary site and the urothelial origin of malignancy. Tumors expressing, both CK7 and CK20, frequently but not always, correspond to UCs of the bladder; however, in one of our cases, CK20 was negative (Table 1). The percentage of positive CK20 in UC ranges from 15% to 97% in different studies [16]. Cytokeratin 7 can be used as an indicator for urothelial differentiation, especially in males, since it distinguishes urothelial malignancies from prostate cancer.

GATA binding protein 3 (GATA-3) is a transcription factor which belongs to a distinct family of tumor suppressor genes. It is involved in human cancer cell growth and differentiation, and plays an important role in cell proliferation and apoptosis. GATA-3 immunocytochemical negativity cannot also exclude UC especially when UC is derived from flat lesion (carcinoma in situ).

Moreover, although the monoclonal antibody to GATA-3 has been shown to be significantly expressed in most urothelial carcinoma variants, other tumors are known to express GATA-3 significantly i.e. breast carcinoma (lobular and ductal), basal cell carcinoma of the skin, malignant mesothelioma, paraganglioma, and chromophobe renal call carcinoma, according to the study of Miettinen et al. [17].

In terms of metastatic UC to the lung, GATA-3 is a useful marker as the most common problem is discriminating UC from a primary pulmonary squamous cell carcinoma (SCC) [18]. Moreover, distinguishing invasive high-grade UC from other carcinomas occuring in the genitourinary tract, such as high-grade prostatic adenocarcinoma, spread from an anal SCC or spread from a uterine cervical SCC, can be challenging.

In addition GATA-3 level of expression was found to be an independent factor predicting cancer reccurence. Furthermore, strong GATA-3 expression was noted to have an effect on tumor size in patients with UC [19, 20].

Carcinoma Antigen 19-9 (CA 19-9) is a blood group-related carbohydrate antigen that reacts specifically with sialyl Lewis-containing glycolip. Blood group antigens are a group of carbohydrate structures bound to membrane lipids or proteins of red cells and certain epithelial tissues, including urothelium [8]. In normal urothelium, CA 19-9 is generally present in the cytoplasm, and it is reported to be expressed on cell membranes in non-invasive carcinomas, and in the cytoplasm of invasive cancer cells [14, 21]. CA 19-9 has been

clinically used as a tumor marker for pancreatic cancer, colorectal cancer; there is limited data on the use of CA 19-9 as a tumor marker in bladder cancer [22]. Recently, it has been reported that increased levels of urinary or serum CA 19-9 indicate poor prognosis in patients with bladder cancer [9, 10, 23, 24].

In the present report, all three cases exhibited markedly elevated serum CA 19-9 levels, so that, clinically, a pancreatic primary was suspected. However, the cytomorphologic findings combined with the immunohistochemical profile of the tumor cells and the clinical history of the patients permitted the diagnosis of metastatic UC of the bladder. Positive CA 19-9 cytoplasmic immunoreactivity was actually found in a number of carcinoma cells in all cases. There are rare reports in the English literature about combined serum and immunohistochemical detection of CA 19-9 in urothelial cancer; Camain and colleagues [17] reported the case of a 75-year-old woman with liver metastasis from urothelial carcinoma and marked increase in serum CA 19-9 levels [25]. Kuroda et al. reported a case of combined small cell and conventional urothelial carcinoma of the bladder producing high levels of serum CA 19-9; in the latter, CA19-9 expression was confirmed in cancer cells of both tumor components [26]. The mechanism of enhanced CA 19-9 expression in bladder cancer is unclear. Increased production by tumor cells and decreased clearance due to urinary tract occlusion might be the cause. In a study by Nagao et al. overproduction of CA 19-9 by the bladder cancer cells was postulated to be the reason for the increased CA 19-9 urinary levels found in bladder cancer patients [27].

In conclusion, our cases reinforce the view that metastatic urothelial carcinoma may overexpress CA 19-9 leading to an elevation of the serum CA 19-9 levels, thus highlighting the ominous significance of serum CA 19-9 elevation in patients with bladder cancer. Immunohistochemical detection of CA 19-9 in cytological material from metastatic tumors of unknown origin may have some diagnostic significance indicating, in the proper setting, an urothelial primary, among other sites

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