Differential diagnosis of traditional serrated adenomas and tubulovillous adenomas : a compartmental morphologic and immunohistochemical analysis

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Abstract

Aim **: Traditional serrated adenomas are the rarest member of the serrated polyps, that have endoscopic and morphologic similarities with conventional adenomas, tubulovillous adenomas, in particular. We aimed to compare the histopathologic and immunohistochemical features of TSAs showing overt dysplasia with conventional TVAs in a compartmental manner using digitalized images.**

Patients and methods **: For 25 TSAs and 25 TVAs, extent of the morphologic features including cytoplasmic eosinophilia, mid-zonal nuclei, ECFs, slit-like serration, brush border, gastric foveolar-like epithelium and goblet cells were evaluated. Immunohistochemistry was perfomed using primary antibodies including CK7, CK20, MUC2, MUC5AC, MUC6, B-catenin, Ki67, p53, p16, MLH1, MSH2, MSH6 and PMS2.**

Results **: Eosinophilic cells, mid-zonal nuclei, slit-like serration and ECF were significantly more extensive in TSAs compared to TVAs (p<0,001) while gastric epithelium was also more extensive in TSA cohort with a lower significance (p<0,01). Cut-offs for these features yielding the highest sensitivity and specificity in discriminating TSAs from TVAs were determined ; mid-zonal nucleus resulted as the best discriminating histopathologic feature (100%, 92%) followed by eosinophilia (88%, 92%),and slit-like serration (84%, 92%) with highest sensitivity and specificities, respectively. Compartmental immunohistochemical evaluation revealed that CK20 and CK7 were mainly expressed in ECF while MUC5AC together with CK7 were found in epithelial compartment more frequently in TSAs compared to TVAs. P16 was more common in TSAs in all compartments whereas Ki67 and p53 were restricted to dysplastic compartments in both polyp groups.**

Conclusions **: The present study demonstrated that mid-zonal nuclei, eosinophilic cells and slit-like serration followed by ECF proved to be the most discriminatory features for TSAs.The correct diagnosis of TSAs will allow to develop appropriate treatment and follow up modalities which seem to be crucial as their progression rate may be different from TVAs.** (Acta gastroenterol. belg., **2020**, 83, **549-556**).

Key words : colonic polyps, traditional serrated adenoma, tubulovillous adenoma, overlap histology.

Introduction

Majority of colorectal carcinomas develop through conventional adenoma-carcinoma sequence while approximately 20% to 30% follow the serrated neoplasia pathway in which sessile serrated adenomas/polyps (SSAs) and traditional serrated adenomas (TSAs) are the potential precursor lesions (1-3). TSA was first described by Longacre and Fenoglio-Preiser (4) as serrated adenoma without a prefix, and later the histologic features were revisited and it was renamed as traditional serrated adenoma by Torlakovic et al. (5) to distinguish it from sessile serrated adenoma. TSAs, the least common of the serrated polyps, are encountered in fewer than 1% of colonoscopies and frequently favour a distal location. Grossly, they tend to have a protuberant exophytic configuration while microscopically complex crypt architecture with characteristic ectopic crypt foci (ECF), slit-like serration and centrally placed, pencillate nuclei (variably termed as “mid-zonal” nuclei) with intense cytoplasmic eosinophilia are the defining features. However, characteristic cytological features, particularly, intense eosinophilic cytoplasm and pencillate nuclei can be found in other serrated polyps as well as conventional adenomas including villous adenomas (VAs) and TVAs (6). On similar grounds, ECFs can be found in VA/TVAs with a reported frequency of 32,5%-100% (7,8). Furthermore, a considerable proportion of TSAs, especially small and flat ones were shown to lack ECFs (9) in several studies.

TSAs were considered as dysplastic ab initio (5), though, presently, some experts believe that TSAs are not overtly dysplastic (10) as there is little mitotic activity, if any, in the eosinophilic epithelium which contains bland-looking nuclei different from the conventional adenomatous epithelium. On the other hand, overt dysplasia, either adenomatous or serrated may be seen in TSAs (1). When adenomatous dysplasia predominates, it confers a conventional TVA appearance on TSA, recently named as “serrated TVA” (STVA) (11) which complicates the distinction of these two types of polyps, further.

There are a few studies in the literature regarding immunohistochemical features of TSAs which may serve useful in their differentiation from TVAs (9, 12,13). Furthermore, immunephenotypic findings would highlight dysplastic foci within TSAs causing resemblance to TVAs. In this context, cytokeratin profile in terms of CK7/CK20 expression proved useful in their differentiation as CK7 positivity was associated with serrated phenotype (13). Characteristic eosinophilic cells with mid-zonal pencillate nuclei in TSAs showed no or scarce Kİ67 positivity which was restricted to ECF

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and dysplastic areas while conventional adenomatous epithelial lining of TVAs showed widespread expression.

Although, confusion still exists regarding the differential diagnosis of these polyps, they potentially represent two different pathways of colorectal neoplasia with different clinical and surveillance implications (14). Therefore, in clinical practice it is important to distinguish, though challenging due to both being exophytic, papillary, and villiform lesions, TSAs from TVAs.

We, hereby, aimed to compare the histopathologic and immunohistochemical features of TSAs showing overt dysplasia with conventional TVAs in a compartmental manner using digitalized images to facilitate simultaneous comparisons and to further comment on the concept of STVA.

Material and methods

Patients and samples

In the present study 25 TSAs and 25 TVAs diagnosed between 2015 and 2018, by two gastrointestinal pathologists (AE &BS) were evaluated. The histological inclusion criteria for TSAs were based on previously published features (1,4,5) comprising typical cytology of abundant brightly eosinophilic cytoplasm with centrally placed pencillate nuclei, complex tubulovillous architecture with slit-like epithelial serration and ECFs. All TVAs were characterized primarily by their tubular and villous architecture with the cytology, displaying adenomatous epithelium characterized by pseudostratified epithelial cells with crowded hyperchromatic oval nuclei showing frequent mitoses. The polyps were removed by polypectomy, endoscopic mucosal resection or surgical resection depending on their size, number and location. Clinicopathologic data including patient age, gender, polyp size and location were collected from automated hospital database. Polyp location was grouped as proximal (proximal to the splenic flexure) and distal colon (distal to the splenic flexure including rectum). Both H&E and immunostained slides were scanned to obtain digitalized images using a digital scanner (3D Histech Pannoramic 250 flash3) along with Case Viewer program and further histopathological examination was performed on digital images.

Histopathologic examination

For each polyp, extent of the morphologic features including cytoplasmic eosinophilia, centrally placed pencillate nuclei, ECFs, slit-like serration, brush border, gastric foveolar-like epithelium containing apical mucin droplets and goblet cells were evaluated on digitalized H&E images and presented as percentages. Histopathologic features defining TSA are demonstrated in figure 1. The presence, grade and type of dysplasia (adenomatous or serrated) and association with carcinoma were also noted in each polyp. In TVAs high grade adenomatous dysplasia and association with carcinoma were recorded while those without such foci were considered as low grade dysplastic by definition. Presence and extent of adenomatous dysplasia and/or serrated dysplasia and carcinoma were also determined in TSAs. The polyps were examined for the coexistence of HP, SSA and conventional adenomas admixed and/or adjacent to TSAs or as precursor lesions. Growth pattern of the polyps either exophytic or flat ; and the presence of a stalk were also noted.

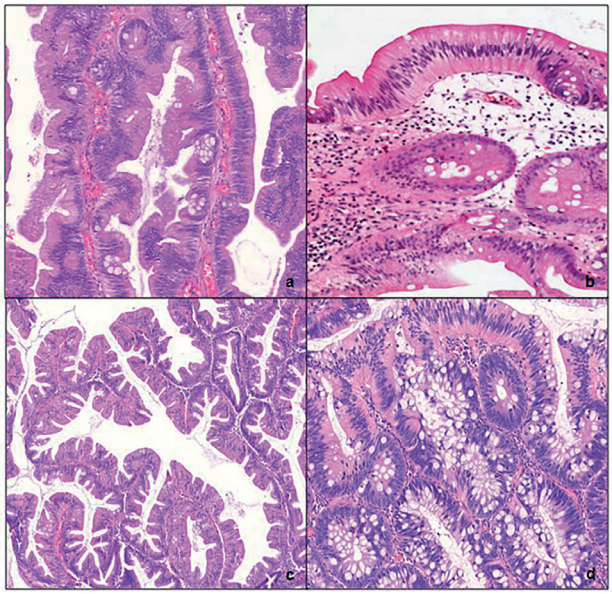

Figure 1. — Defining morphologic features of TSA (all H&E) : a) Numerous ECFs with horizontal orientation towards muscularis mucosa (x50), b) Centrally placed nuclei within high columnar eosinophilic epithelium (x100), c) Crypts showing slit-like luminal serration (x30), and d) Low grade adenomatous dysplasia in TSA (x100).

Immunohistochemical examination

Immunohistochemistry (IHC) was perfomed on 4 micron-thick tissue sections prepared from formalin-fixed parafin-embedded (FFPE) tissue blocks using primary antibodies including, CK7 (clone : OV-TL 12/30, 1 :200, Cell M), CK20 (clone : Ks20.8, 1 :200, Cell M), MUC2 (clone : MRQ-18 ; RTU, Cell M), MUC5AC (clone : 1-13M1, 1 :150, NeoMarkers), MUC6 (clone : CLH5 ; 1 :50, NeoMarkers), B-catenin (clone : E247, 1 :150, NeoMarkers), Ki67 (clone : 30-9,RTU, Ventana), p53 (clone : DO-7+BP53-12, 1 :250, NeoMarkers), p16 (clone : E6H4, RTU, Ventana), MLH1 (clone : M1,RTU, Cell M), MSH2 (clone : G-219-1129, RTU, Cell M), MSH6 (clone : CLH5, 1 :50, NeoMarkers)and PMS2 (clone : EPR.3947 ; RTU, Ventana). All IHC procedures were automatically conducted using a BenchMark XT immunostainer (Ventana Medical Systems, Tucson, AZ) according to the manufacturer's protocol. CK7, CK20, MUC2, MUC5AC, MUC6, CDX2 and p16 were assessed for intensity and extent of staining in the following

Table 1. — **Compartmental histopathologic analysis of the polyp groups**

	Eosinophilia	Mid-zonal nucleus	Slit-like Serration	ECF	Brush Border	Gastric fov.-like epithelium	Goblet cells
TSA (n:25) Frequency (%)	100	100	100	100	100	100	100
Extent (%) Median (Min-Max)	70 (30-90)	50 (20-80)	60 (10-90)	60 (20-80)	50 (20-70)	20 (20-70)	40 (20-70)
TVA (n:25) Frequency (%)	96	44	52	100	100	88	96
Extent (%) Median (Min-Max)	20 (0-60)	0 (0-40)	10 (0-40)	20 (10-40)	30 (20-50)	20 (0-50)	30 (0-80)
p value	p<0,001	p<0,001	p<0,001	p<0,001	NS	p<0,01	NS

Table 2. — **Cut-offs of the histologic features in distinction TSAs from TVAs**

Morphologic feature	Cut-off %	Sens %	Spec %	PPV %	NPV %
Eosinophilia	45	88	92	91	88
Mid-zonal nucleus	15	100	92	92	100
Slit-like serration	25	84	92	91	85
ECF	45	72	100	100	78
Gastric epithelium	15	100	40	62	100

compartments ; basal zone, eosinophilic cells, ECFs, gastric-foveolar epithelium, goblet cells, dysplastic and carcinomatous foci using H&E images as reference to identify the compartments. Overall intensity and extent of staining were also noted. Intensity was scored as 0-3 (0 : no staining, 1 : weak staining, 2 : moderate staining, 3 : strong staining) and extent as 0-3 (0 : no staining, 1 : 1-33% of cells, 2 : 34-66% of cells, 3 : >66% of cells), on an arbitrary scale. A final score was determined by multiplying the intensity and extent scores (minimum score :0, maximum :9). Ki67 staining was assessed only for extent of staining in each compartment described above as only strong nuclear staining is accepted as positive for this marker. Extent of staining with strong nuclear expression of p53 was evaluated in dysplastic and/or carcinoma foci. Nuclear B-catenin expression was assessed in dysplastic and carcinoma foci while membranous and cytoplasmic stainings were also noted. Loss of MLH1, MSH2, MSH6 and PMS2 expression was noted when one or more clusters of tumor cells or all tumor cells were negative with these nuclear markers.

Statistical analysis

Statistical analysis was performed by Chi-Squared test for frequencies and Mann-Whitney test for comparisons of extent of morphologic and immunohistochemical features. Histopathologic variables were tested for their discriminatory value with ROC curve analysis and those with discriminatory value were further assessed with Youden index. Differences were considered significant at $p<0.05$ level.

Results

A total of 50 cases comprising 25 TSAs and 25 TVAs were included in the study. Mean age of the patients was 60,6 years (ranging between 39 and 79) in the TSA group with a male predominance (M/F : 15/10) whereas in the TVA group, mean age was 64,8 (ranging between 30 and 84) with a similar male predominance (M/F : 15/10). All TSAs were pedunculated polypoid lesions resembling conventional adenomas endoscopically. Median polyp size was 2,6 cm (ranging between 0,2 cm and 4,8 cm) in TSAs and 2,36 cm in TVAs (ranging between 0,8 cm and 4,5 cm). Majority of the polyps were located in the distal colon while proximal location was observed in 20% of TSAs and 18,7% of TVAs. Three TSAs and two TVAs were removed by resection while the rest were excised by polypectomy. All TSAs and TVAs were exophytic with stalks except three TSAs which were sampled superficially.

Histologically, all TSAs (25/25 ; 100%) contained eosinophilic cells, centrally placed pencillate nuclei, brush border, slit-like serration, ECFs, gastric/foveolar-like epithelium and goblet cells. ECF and brush border were also present in all (25/25 ; 100%) TVAs, followed by eosinophilic cells, goblet cells, and gastric/foveolar-like epithelium which were found in 96%, 96%, and 88% of the polyps, respectively. Eosinophilic cells, centrally placed pencillate nuclei, slit-like serration and ECF were significantly more extensive in TSAs compared to TVAs (p<0,001) while gastric-like epithelium was also more extensive in TSA cohort with a lower significance (p<0,01). Results of compartmental histopathologic analysis is summarized in Table 1.

Dysplasia was present in 23 (92%) TSAs, 14 (56%) of which showed focal low grade adenomatous dysplasia while 9 (36%) showed high grade dysplasia (3 serrated+6 adenomatous). Five TSAs contained intramucosal carcinoma, all with high grade adenomatous dysplasia in the neighboring areas. In the TVA cohort high grade

dysplasia was observed in 9 (36%) and intramucosal carcinoma was present in 5 (20%) polyps. Precursor lesion was present only in three TSAs ; two sessile serrated adenoma/polyp and one hyperplastic polyp, while no such lesion was found in TVA cohort.

After determining the extent of histopathologic features with statistical significance, ROC curve analysis was employed to identify the cut-offs for these features yielding the highest sensitivity and specificity in discriminating TSAs from TVAs (Table 2). The cut-offs for centrally placed pencillate nuclei and gastric epithelium were both 15%, 25% for slit-like serration and 45% for both eosinophilia and ECF. Accordingly, mid-zonal nucleus resulted as the best discriminating histopathologic feature (100%, 92%) followed by eosinophilia (88%, 92%), and slit-like serration (84%, 92%) with highest sensitivity and specificities, respectively.

Combination of histopathologic features with high discriminatory values (eosinophilia, mid-zonal nucleus, and slit-like serration depicted as 1, 2, and 3 in figure 2) yielded similar results in terms of sensitivities and specificities.

Compartmental Immunohistochemical Analysis

Cytokeratin profiles showed that CK20 was the most widely expressed marker in both TSAs and TVAs in all compartments. When we examined the intensity and extent of stainings in TSA group ; CK20 was predominantly expressed in eosinophilic cell compartment with a mean value of 7±2,85 which was significantly higher than TVAs (mean : 2,84±2,51) ($p<0,001$). CK20 expression, in ECFs, though lower than eosinophilic cells, was statistically higher in TSAs compared to TVAs ($p<0,01$). There was no statistical significant difference between the two cohorts foroverall CK7 expression ; but the intensity and extent of staining of CK7 was significantly higher in gastric foveolar-like epithelial compartment ($p<0,01$) and ECFs ($p<0,05$) in TSAs. MUC2 expression in goblet cell compartment ($p<0,05$) and MUC5AC expression in the goblet cells ($p<0.001$), basal zone and gastric/foveolar-like epithelium ($p<0,01$) were significantly higher in TSAs compared to TVA group while Ki67 was mostly expressed in dysplasia and carcinoma compartments, followed by ECFs, goblet cells and basal zone, and scarce or none in eosinophilic cells. Except gastric/foveolar-like epithelium compartment, p16 expression was significantly higher in all other compartments in TSA group. Nuclear expression of p53 was evaluated in dysplastic foci and/or carcinoma foci, and there was no statistically significant diffence between the two groups. Expression of nuclear B-catenin was observed in 5 TVAs, and one TSA in advanced neoplastic areas of the polyps. MLH1, MSH2, MSH6 and PMS2 were preserved in all polyps even in the advanced areas in both groups. The results of compartmental immunohistochemical analysis are summarized in Table 3.

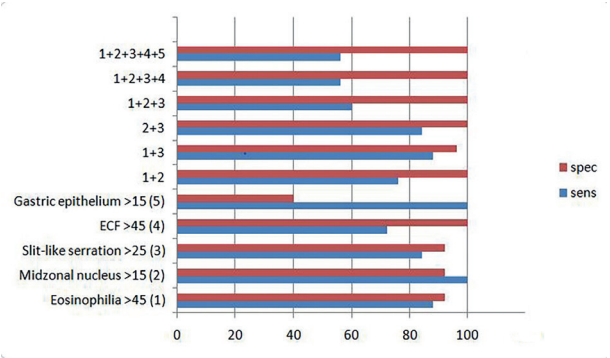

Figure 2. — The diagnostic value of individual variables and their combinations for differentiating TSAs from TVAs.

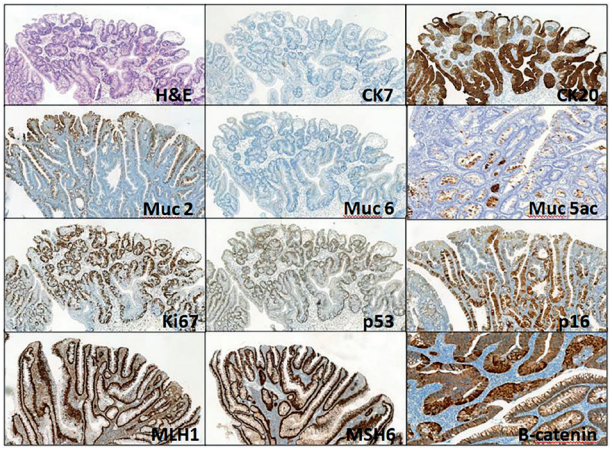

Figure 3. — Immunohistochemical features of TSA (see IHC markers inserted on each image with x50 magnifications except for MUC5AC and B-catenin which are both x100 magnified).

Discussion

Among the serrated polyps, the origin, morphologic features, molecular modifications and immunohistochemical alterations of TSAs are the least understood ; probably due to the rarity and lack of definite morphological criterias. Although typical cytology, ectopic crypt formations, slit-like luminal serrations, and protuberant or villiform growth have proven useful, absolute diagnostic criteria is difficult to define, because these features may also be observed in other colorectal polyps, particularly TVAs. To ascertain the degree of histological overlap between conventional VA/TVAs and TSA, and also to determine the most useful histopathologic and immunophenotypic features to differentiate these polyps were the main aims of this study.

The results of the present study showed that mid-zonal nucleus proved to be the most discriminatory feature with the highest sensitivity and specificity, followed by eosinophilia and slit-like serration in distinguishing TSAs from TVAs. Moreover, we found that all diagnostic histopathologic features of TSAs were also present in the TVA cohort. Main difference, however, was the extent of

Table 3. — **Immunohistochemical results of markers that evaluated compartmentally**

Antibody	**TSA (n:25) Mean±SD**	**TVA (n:25) Mean±SD**	**p value**
CK7			
Overall	0,92±1,25	0,36±0,48	
Basal zone	0,22±0,6	0,24±0,43	
Eosinophilic cells	0,68±1,46	0,04±0,2	
ECF	0,16±0,37	0,04±0,2	p<0,05
Gastric/foveolar cells	1,76±2,84	0,16±0,37	p<0,01
Goblet cells	0,2±0,57	0,12±0,33	
Dysplastic/neoplastic area	0,24±0,83	0,12±0,33	
CK20			
Overall	5,92±2,76	5,52±2,4	
Basal zone	1,9±2,13	1,44±1,55	
Eosinophilic cells	7±2,85	2,84±2,51	p<0,001
ECF	3,12±2,45	1,28±1,1	p<0,01
Gastric/foveolar cells	2,4±2,5	2±2,9	
Goblet cells	4,72±3,18	3,6±2,5	
Dysplastic/neoplastic area	3,72±2,7	3,28±2,17	
MUC2			
Overall	3±1,68	4,04±1,45	p<0,05
Basal zone	2,72±2,56	1,84±1,54	
Eosinophilic cells	2,48±2,58	1,04±1,2	
ECF	1,68±1,51	0,84±0,37	
Gastric/foveolar cells	0,28±0,45	0,8±1,2	
Goblet cells	4,64±2,67	3,16±2,32	p<0,05
Dysplastic/neoplastic area	2,56±2,41	2,76±2,01	
MUC5AC			
Overall	2,52±1,47	0,96±0,53	p<0,001
Basal zone	0,86±1,28	0,8±0,27	p<0,01
Eosinophilic cells	0,8±1,58	0,4±1,11	
ECF	0,48±1,22	0,16±0,37	
Gastric/foveolar cells	2,56±2,02	0,92±1,25	p<0,01
Goblet cells	2,16±1,49	0,72±0,67	p<0,001
Dysplastic/neoplastic area	0,64±1,31	0,48±1,12	
p16			
Overall	2,84±1,37	1,32±0,69	p<0,001
Basal zone	1,63±1,73	0,48±0,5	p<0,05
Eosinophilic cells	1,68±1,37	0,68±0,85	p<0,01
ECF	1,24±1,3	0,48±0,5	p<0,05
Gastric/foveolar cells	0,36±0,56	0,2±0,40	
Goblet cells	1,36±1,11	1,12±0,92	p<0,01
Dysplastic/neoplastic area	2,2±1,68	1,66±1,45	p<0,05
Kİ67			
Basal zone	1,50±0,67	1,11±0,33	p<0,05
Eosinophilic cells	0,84±0,55	0,8±0,57	
ECF	1,56±0,65	1,64±0,63	
Gastric/foveolar cells	1,16±0,62	1,16±0,62	
Goblet cells	1,72±0,54	1,12±0,6	p<0,01
Dysplastic/neoplastic area	2,72±0,45	2,4±0,5	p<0,05

these features which were extensively present in TSAs in contrast to TVAs in which they were only focally present. Mid-zonal nucleus was observed in all TSAs covering 50% of the epithelial compartment within the polyps while majority of TVAs did not contain mid-zonal nucleus which, when found was present only focally. Similarly, slit-like serration and cytoplasmic eosinophilia were present extensively in the TSAs covering 60% and 70% of the polyps, respectively. Though, found in the majority of TVAs, they were present only focally covering 10% and 20% of the polyps, respectively. Combination of these histopathologic features did not improve the sensitivity and specificity.

WHO Classification of Tumors of the Digestive Tract emphasizes ECFs and villiform, protuberant growth pattern In the diagnosis of TSAs (1) while typical cytology, comprising centrally placed, palisated, pencillate nuclei with intensely eosinophilic cytoplasm, though common, is not a prerequisite for diagnosis. On similar grounds, the most reliable and reproducible features including tubulovillous architecture, ECF, slit-like serration and typical cytologic appearance of intensely eosinophilic cytoplasm and centrally placed pencillate nuclei in various combinations were reported in previous publications (9,15,16). In the study of Bettington et al. ; at least two of these features ; i) typical cytology, ii) slit-like serrations and iii) ECFs, with at least one feature being present in >50% of the polyps were accepted as inclusion criteria for TSAs (9). In a recent study, Hiromoto et al. classified a polyp as a TSA, when all these feature with exophytic architecture were present in >50% of the polyps (17).

Due to the similarities between TSAs and conventional adenomas, particularly TVAs, morphologic features

defining TSAs were also evaluated in TVAs by several investigators. Bakhtiari et al. showed that none of the conventional adenomas had luminal slit-like serrations and only 10% of the cases had focal cytoplasmic eosinophilia (18). Herein, slit-like serration deserves a short description as it is quite different than the serration seen in other serrated polyps. It is in the form of deep clefts and slit-like spaces leading to broad luminal fronds that project in the gland lumen imparting a jigsaw puzzle like appearance. Together with cytoplasmic eosinophilia these characteristic serrations have recently been described as "enteric metaplasia" due to their resemblance to normal small intestinal villous epithelium (19). Not necessarily slit-like, but epithelial serrations can also be seen in conventional adenomas (20). Prominent brush border is noted on the surface, further enhancing the resemblance to intestinal epithelium. Complicating the issue further, Bettington has recently described a "serrated" TVA, which occurs more frequently in a proximal location, and morphologically resembles a conventional TVA with prominent serrations (at least > 50% of the polyp) (11). However, others argue that the "undulating" or "mazelike" serrations described in "serrated" TVA may possibly be secondary to mechanical compression due to luminal spatial constriction and thus, are quite different from the slit-like serration seen in TSAs (21).

ECFs, small, nest like structures of abnormally developed crypts losing their anchor on the muscularis mucosa were initially thought to be pathognomonic for TSAs (1,10) but later ECFs were reported to be encountered in other types of serrated polyps and also in conventional adenomas (6,7). ECFs were found in 53,8% of TVAs and 100% of VAs in the study of Väyrynen SA et al. (7). Furthermore, notable proportions of TSAs, especially small and flat ones were reported to lack ECFs by some investigators (9). In our study, ECFs were a prominent feature of TSAs while they were present only focally in TVAs.

Although, some TSAs arise in a precursor polyp, especially microvesicular hyperplastic polyps or sessile serrated adenomas, some of them develop de novo. The incidence of precursor lesions was reported to be 52.3% in the study by Kim et al. (22). In the present study only three TSAs had a precursor lesion one of which was HP and others were SSA/P.

Two major patterns of dysplasia were reported in TSAs ; serrated dysplasia and conventional adenomatous dysplasia (1). Dysplasia was present in 92% of TSAs in our cohort, mostly low grade, though some showed serrated high grade dysplasia while few cases with intramucosal carcinoma had conventional adenomatous dysplasia. We strongly believe that polyps creating confusion with TVAs are the TSAs which contain foci of low grade conventional adenomatous dysplasia resembling adenomatous epithelium. Indeed, mixed histologic features have been demonstrated in some TSAs including adenomatous epithelium which led to speculations that a spectrum of polyps do exist (12). Considering the similarity of certain genes taking part in different colorectal cancer pathways, occurrence of morphological overlaps should not be surprising. Also phenotypic alterations may occur in a polyp as it accumulates genetic changes, evolving from serrated pathway to conventional pathway. It is well known that a shift from membranous to nuclear staining of B-catenin is indicative of Wnt signaling pathway activation in conventional adenomas. Although recent studies have reported increased nuclear B-catenin expression in advanced areas of TSAs (9,17) ; only one TSA and 5 TVAs had nuclear B-catenin expression in advanced areas of the polyps in our study. The sTVAs defined by Bettington, however, showed more frequent nuclear B-catenin expression also reflecting WNT pathway activation which is morphologically characterized by adenomatous epithelium (11). It seems that there is a spectrum of lesions starting with a TSA (with slit-like serration, ECFs, typical cytology) evolving into a TSA with conventional dysplasia and, eventually to conventional adenoma (12,18). Therefore, while typical histologic features including centrally placed nuclei, eosinophilic cytoplasm, slit-like serrations and ECFs lead to a diagnosis of TSA, presence of adenomatous dysplasia should raise the possibility of a transition to TVA which should be mentioned in the pathology report.

Compartmental immunohistochemical analysis performed in the present study has clearly facilitated our diagnosis of dysplasia and/or neoplasia within each polyp. In accordance with this view, Ki67 was predominantly expressed in dysplasia and carcinoma compartments as expected, followed by ECFs and basal zone while eosinophilic cells showed only scarce positivity, if any. Although, cytoplasmic eosinophilia in TSAs has been considered as part of dysplastic phenotype by some authors, others believe that these cells should be regarded as metaplastic or even senescent (2, 19) due to low proliferative activity along with lack of APC mutations. Our immunohistochemical findings are in accordance with the latter view (19).

In a recent study, Tatsumi et al. reported that serrated adenomas and adenocarcinomas arising in serrated adenomas in the colorectum showed CK7+/CK20+ profile suggesting that CK7 could be a marker for the serrated pathway (13). Although, there was no statistically significant difference between the two cohorts in overall intensity and extent of staining in CK7 in the present study ; compartmental assesment revealed that CK7 expression was significantly higher in TSAs, particularly in gastric foveolar-like epithelium. This finding was consistent with Tatsumi's suggestion regarding the relation of CK7 expression with serrated morphology. CK 20, on the other hand, the most extensively expressed marker in both TSAs and TVAs, was predominantly present in eosinophilic cell compartment in the TSA group. MUC gene expressions were in accordance with CK pattern as MUC2 was widely expressed in goblet cells ; but low in gastric/foveolar-like epithelium and ECF components

whereas MUC5AC was more commonly expressed in TSAs with a predilection to gastric/foveolar-like epithelium a finding which correlated with the results of a previous study (24). Gibson et al. (25) found that the expression levels of MUC1, MUC2, and MUC5AC were not significantly different between serrated polyp subgroups or between serrated polyps and carcinomas while in Renaud's study MUC2 and MUC5AC expressions were more frequent in serrated polyps than in carcinomas (24). We, on the other hand, found lower MUC5AC expression in the neoplastic compartments of both TSAs and TVAs, although MUC2 did not show any difference between the compartments.

Despite the varying expression profile of p16 in TSAs, we and others believe that p16 overexpression is a common feature in the neoplastic progression of serrated lesions. Accordingly, except gastric/foveolar-like epithelium compartment, intensity and extent of staining of p16 was higher in TSAs compared to TVAs in all compartments with the highest positivity in dysplastic/neoplastic areas in our study. This is consistent with Kriegl et al.'s results while is in contrast with Bettington who found loss of p16 staining in the advanced areas of 55% of BRAF mutant TSAs (9,26).

One of the molecular characteristics found in sessile serrated adenoma/polyp with dysplasia is MSI as a consequence of MLH1 gene silencing due to promoter hypermethylation (27). Loss of MLH-1 expression by immunohistochemistry is a distinctive feature of dysplastic SSA/Ps, particularly those with minimal deviation dysplasia (14) while those with serrated and/or adenomatous dysplasia do not show this molecular aberration. This, also, is not a common feature of TSAs which is in accordance with our findings. We found no MSI in none of the polyps in our cohort including the TVA group.

In conclusion, the results of the present study have clearly demonstrated that TSAs and TVAs have morphological similarities leading to diagnostic difficulty in clinical practice. This is particularly important as endoscopic features of these two types of polyps are almost identical with their protuberant growth pattern. However, characteristic epithelial lining of TSAs shows features of non-dysplastic senescent epithelial cells in contrast to low-grade dysplastic adenomatous epithelium found in TVAs. Compartmental morphologic and immunohistochemical analysis highlighted these differential features in both polyp types. Our results showed that mid-zonal nuclei, eosinophilic cells and slit-like serration followed by ECF proved to be the most discriminatory features for TSAs. Immunohistochemical analysis, when perfomed in a compartmental manner, may facilitate the recognition of gastric foveolar-like epithelium by CK7 and MUC5AC, ECF by accumulation of Ki67, senescent eosinophilic cells by CK20 positivity and lack of Ki67. While nuclear B-catenin expression was a more common finding in TVAs, in the advanced areas, TSAs showed p16 overexpression in such foci.

Currently the treatment and follow-up strategy of TSAs are based on those of conventional adenomas as their progression rate and patterns are still largely unknown. On similar grounds, macroscopic handling and reporting of these polyps follow those of conventional adenomas. A recent review highlights the main points of handling and reporting of malignant colorectal polyps regardless of histologic type. In addition to the known risk factors of malignant polyps such as polyps size, location, depth of invasion, lymphovascular invasion, tumour budding, the authors recommend to include status of the muscularis mucosa, the lateral mucosal margin, and tumour type (28). Therefore, until a different strategy is proposed, the above approach seems to be appropriate for malignant serrated polyps including TSAs.

Conflict of interest

The authors declare no conflict of interest.

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