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ORIGINAL ARTICLE

Defined morphological criteria allow reliable diagnosis of colorectal serrated polyps and predict polyp genetics

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Abstract Criteria for the diagnosis of serrated colorectal lesions (hyperplastic polyp, sessile serrated adenoma without or with dysplasia—which we called mixed polyp—and traditional serrated adenoma) for which consensus has been reached should be validated for applicability in daily practice in terms of inter-observer reproducibility and their association with clinical features and (epi)genetic events. A study set was created from a consecutive series of colorectal polyps (n=1,926) by selecting all sessile serrated adenomas, traditional serrated adenomas and mixed polyps. We added consecutive series of hyperplastic polyps, classical adenomas and normal mucosa samples for a total of 200 specimens. With this series, we conducted an inter-observer study, encompassing ten pathologists with gastrointestinal pathology experience from five European countries, in three rounds in which all cases were microscopically evaluated. An assessment of single morphological criteria was included, and these were correlated with clinical parameters and the mutation status of *KRAS*, *BRAF* and *PIK3CA* and the methylation status of *MLH1*. Gender, age and localisation were significantly associated with certain types of lesions. Kappa statistics revealed moderate to good inter-observer agreement for polyp classification (κ = 0.56 to 0.63), but for single criteria, this varied considerably (κ = 0.06 to 0.82). *BRAF* mutations were frequently found in hyperplastic polyps (86 %, 62/72) and sessile serrated adenomas (80 %, 41/51). *KRAS* mutations occurred more frequently in traditional serrated adenomas (78 %, 7/9) and less so in classical adenomas (20 %, 10/51). Single morphological criteria for sessile serrated

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adenomas showed significant correlation with *BRAF* mutation (all $p \leq 0.001$), and those for classical adenomas or traditional serrated adenoma correlated significantly with *KRAS* mutation (all $p < 0.001$). Therefore, single well-defined morphological criteria are predictive for genetic alterations in colorectal polyps.

Keywords Colorectal polyps · Serrated polyps · SSA · TSA · *BRAF* · *KRAS* · Inter-observer study

Introduction

Identifying precursor lesions of colorectal cancer is a challenge for cancer prevention programmes [12, 19]. The classical adenoma-carcinoma sequence, predicting that carcinomas arise from treatable adenomas, has served as scientific basis for colorectal cancer prevention programmes. Interval carcinomas particularly in the right colon have challenged this approach [3]. During recent decades, new molecular pathways involved in colorectal carcinogenesis have been discovered. One of these is the serrated pathway, which is associated with a distinctive subset of colorectal carcinomas [21–23].

Convincing evidence for this new pathway is derived from recent whole genome sequencing, which has unveiled distinctive colorectal cancer subtypes with molecular and morphological homology with sessile serrated adenomas (SSA) [8]. Although these findings have firmly established the existence of a serrated pathway, the morphological criteria currently defined and applied to identify and classify serrated lesions in the colorectum, which include hyperplastic polyps (HP), sessile serrated adenoma (SSA), traditional serrated adenoma (TSA) and mixed polyps (MP), the latter renamed sessile serrated adenoma with dysplasia (SSA-D) in the 2010 WHO classification [37], are still under debate [2, 5, 16, 20].

As long as pathomorphological criteria for diagnosing SSA are not clearly defined, using them as a frame of reference for classifying patterns of molecular aberrations associated with these lesions would be of limited or no value. Diagnosing and grading of dysplasia in Barrett's oesophagus [7] is an example of another area in gastrointestinal pathology for which, beyond establishing consistently described and clearly defined criteria, their intra- and inter-observer reproducibility as indicators of reliability and usability in daily practice are of paramount importance. The criteria proposed and applied for diagnosing serrated colorectal lesions encompass architectural, cytological, immunohistochemical and genetic features but there is still some confusion. We contend that consistent histological analysis is the essential initial step in the assessment of any lesion, which should precede the use of any additional techniques.

The morphological criteria to define serrated lesions used in publications not only include those commonly applied, such as *horizontally oriented crypts* or *ectopic crypt formation*, but also criteria that are not yet widely recognized, such as *surface tufting* or *crypt-stroma-ratio*. Through expert meetings, experience-based, simplified and sufficiently discriminative criteria applicable in daily practice have emerged [1, 34, 37], but these have not all been evaluated scientifically. Our study was conceived when the first papers were published with empirical data as a basis for criteria to diagnose serrated lesions and resulted in the German consensus guidelines published in 2010 [1]. This was prior to the new WHO classification (2010) [37] and to the recommendations from North American expert panels (2012) [34], which as a consequence were not used in the design of the study. Nonetheless, the diagnostic criteria for classifying serrated colorectal lesions are remarkably congruent between these recent classification systems. Inter-observer analysis is the scientific method to critically analyse diagnostic criteria, such as those of the German consensus guidelines, and compare them between different classification proposals.

Our study was designed as an international multicentre inter-observer study involving ten pathologists from five European countries. We chose virtual microscopy rather than serial sections as our approach, as it provides all study participants with absolutely identical visual information. In addition, of all the investigated lesions, *KRAS*, *NRAS*, *BRAF* and *PIK3C* mutation status as well as *MLH1* promoter methylation was established and this was correlated with individual morphological criteria. Through this approach, we found evidence for strong correlations between phenotype and genotype in serrated precursor lesions of colorectal cancer.

Materials and methods

Preparing the study set

A main issue of the study was to reflect daily practice in gastrointestinal pathology. For this reason, we screened a consecutive series of colorectal polyps (n=1,926) diagnosed in a large pathology department specialising in gastrointestinal pathology. Clinical data (gender, age, localisation and size) of the polyps were documented. All lesions diagnosed as serrated adenomas, including TSA (n=7), SSA (n=81) and MP (n=7), were included. To complete the diagnostic spectrum of colorectal lesions, consecutive series of HP (n=50) and classical adenoma (CAD, n=45) were added. The study set was completed by the addition of samples of normal colonic mucosa (n=10) making a total of 200.

The study set was created and prepared for virtual microscopy by two of the authors (K.K. and C.G.), who did not further participate in slide evaluation. Ethical guidelines were respected and all experiments were in accordance with the Declaration of Helsinki.

Scanning and blinding of study set

Slides were scanned with a Zeiss Mirax Scanner with ×400 magnification. Each virtual microscopy slide was prepared without any preselection of regions of interest. To hamper easy recognition of certain lesions, all irrelevant identifying items such as numbers were blinded to the study pathologists and an interval of 4 months was applied between the three rounds. The order of the data files was randomised in each round by the same investigators (K.K. and C.G.). Zeiss Mirax files were shipped on external USB hard drives, providing exactly the same images to each of the study participants (all experienced gastrointestinal pathologist, in alphabetical order A.A., A.H., C.L., A.L., I.D.N., J.R., T.T.R., X.S., M.S. and M.V.). For analysis, the Mirax Viewer Software (3d Histech, Budapest, Hungary) was provided.

Inter-observer study

The study was performed in three rounds, which were denoted panels A–C (Fig. 1). The study meeting between panels B and C included a microscopic training session on a completely different set of colorectal polyps, five in each category, and a review of all these cases. Beyond the diagnosis, additional documentation was provided for panel A to define classical versus serrated dysplasia. In panel B, the quality of the slides was assessed as a separate criterion. In panel C, nine individual criteria were assessed for each lesion.

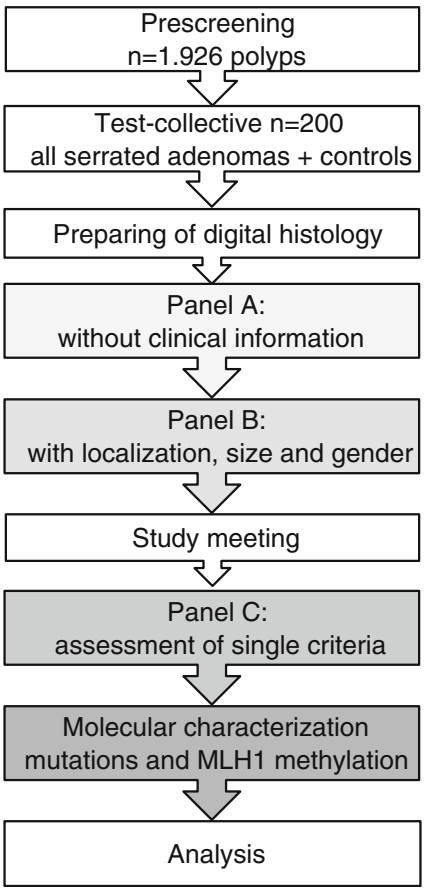

Fig. 1 Design of the inter-observer study in three rounds. Virtual microscopy slides were mixed and redistributed as three separate panels (A–C). Clinical information (gender, age, localisation and size) was given later on (B, C). For panel C, the simplified German consensus criteria were applied and detailed information about the single morphologic criteria in each lesion was assessed. The genetic background was analysed after the last round

Molecular analysis

Two separate 10-μm sections of each lesion were macrodissected and transferred for DNA extraction. DNA extraction was performed using the QIAamp DNA FFPE Kit (Qiagen, Hilden, Germany), according to the manufacturer's recommendations. DNA quantity was measured using an ND-1000 spectrophotometer (Thermo Scientific, Wilmington, USA, DE). DNA quality was assessed by β-globin PCR; primers and protocols are available on request. *KRAS*, *BRAF*, *PIK3AC* and *NRAS* mutations were assessed simultaneously using a multiplex PCR assay, as described previously [29]. *MLH1* methylation analysis was performed after bisulphite treatment of DNA using the Epitect Bisulphite Kit (Qiagen, Hilden, Germany), and pyrosequencing was undertaken using the *MLH1* Pyrosequencing Kit (Qiagen, Hilden, Germany) on a Q24 Pyromark System (Qiagen, Hilden, Germany). This assay covers five CG sites within the CpG island of the *MLH1* promoter. Following criteria defined in the literature, an average methylation grade of >17 % was taken as the cut-off for *MLH1* hypermethylation. Successful *KRAS*, *BRAF*, *PIK3CA* and *NRAS* analysis was achieved for all lesions. Due to advanced DNA degradation by bisulphite treatment, *MLH1* methylation could only be assessed for 150 of the 200 lesions, but in this data set, polyp categories were adequately represented.

Statistics

Study parameters were described either as a mean (± standard deviation) or as a frequency distribution. In some cases, data were summarised for all participants by calculating the median of individual scores (for example, for section quality) or by the number of observers that had assigned a case to a specific category (for example, TSA or SSA).

To obtain a reference diagnosis for each category of polyps and type and grade of dysplasia, a majority vote of the participating pathologists was taken at the last study meeting (panel C). Inter-observer agreement for nominal data (section quality, main diagnosis, specification mixed polyp, serrated and conventional type of dysplasia) and for dichotomous data (TSA, SSA) was assessed by Fleiss' kappa. For main diagnosis, specification MP, serrated type dysplasia and conventional type dysplasia, kappa was assessed separately for the results of each panel meeting.

How single parameters correlated with the main diagnosis was assessed by multinomial models using the R-function

'multinom' [42]. The influence of these parameters on dichotomous rating (these are TSA and SSA) was studied by logistical regression models. In the case of ordinal ratings (serrated and classical type dysplasia), influence parameters were studied by non-parametric analysis of variance.

Furthermore, for Spearman rho correlations, χ^2 tests and sensitivity, specificity calculations were performed to investigate morphologic-genetic correlations. Statistical tests were performed at a significance level of $p \leq 0.05$. All analyses were carried out using the software R (www.r-project.org) and SPSS 19 (IBM, USA, NY).

Results

Colorectal polyps show differences in localisation, size, gender and age

In agreement with previous studies, CADs, HPs and TSAs occurred more frequently in the left colon, whereas SSAs were more often right-sided (Supplemental Fig. 1). However, SSAs were also found in the rectum. Below the age of 40, SSA occurred more often in women than in men. The age-related frequency of CAD increased in both genders. Polyps of >15 mm diameter were more often CAD than SSA (Supplemental Fig. 2).

Inter-observer agreement in the diagnosis of colorectal polyps

The first round (panel A) assessed basic agreement among the study participants using only histomorphological criteria without disclosing clinical parameters and without explicit consensus regarding the diagnostic criteria to be used. Fleiss' kappa achieved a moderate value of $\kappa = 0.56$.

In the second round (panel B), the order of polyps was randomised and clinical data including gender, age, location and size were provided. Fleiss' kappa slightly increased to $\kappa = 0.63$.

In the final round (panel C), we used a completely different training set of polyps, which were discussed at a study meeting (in Erlangen, Germany). General agreement was obtained to apply the consensus criteria from a German working group [1], as listed in Tables 1 and 2. Subsequently, the polyp files were freshly randomised and reassessed by the participants, upon which Fleiss' kappa reached $\kappa = 0.61$, indicating that the consensus criteria maintained the level of reproducibility.

Major challenges in diagnosing colorectal polyps

We analysed voting patterns obtained during panel A (Fig. 2) to identify potential diagnostic challenges. We observed high inter-observer variability for the distinction between HP and SSA, but also between MP and TSA.

We also addressed how well serrated type dysplasia can be distinguished from classical type dysplasia. When using nuclear features to diagnose serrated type dysplasia in SSA, no agreement was obtained ($\kappa = 0.07$), whereas for the diagnosis of classical type dysplasia in CAD, MP or TSA, the agreement was high ($\kappa = 0.75$). As a rule, therefore, SSA should not be considered as dysplastic.

Our inter-observer study indicates that for the vast majority of lesions ($n=159$), the diagnosis was identical from panel A to B to C (Fig. 3). However, for a significant proportion of the lesions ($n=41$), the diagnosis switched for the majority of pathologists (Fig. 4). Of note, the introduction of defined criteria and the mandatory cut-off of at least two affected crypts reduced the number of SSAs and increased the number of HPs. In a multinomial analysis, we identified clinical parameters (age, gender, location and size), section quality but also the pathologist as parameters significantly affecting this diagnostic choice ($p<0.01$). This could be explained by the very strong intra-observer variability, ranging from $\kappa = 0.55$ to $\kappa = 0.77$ (Fleiss' kappa) for every pathologist. Therefore, even when strictly defined criteria are applied to the diagnosis of a specific lesion, the distinction between HP and SSA remains in the realm of the individual pathologist's perception (Fig. 5).

Analysis of single SSA and TSA criteria

Which criteria individual participants used for the diagnosis of SSA and TSA was collected during panel C. Sensitivity and specificity of individual criteria for the reference diagnosis of

Table 1 Specific SSA criteria in panel C: kappa value, dependency from pathologist and quality and frequency in HP, SSA, CAD, TSA and MP

SSA criteria	Basal hyperserration	T-L-shaped crypts	Inverted crypts	Crypt dilatation
κ values	0.43	0.47	0.25	0.38
Pathologist dependency	Yes	Yes	Yes	No
Quality dependency	Yes	Yes	Yes	Yes
HP	8.3 % (6/72)	0.0 % (0/72)	0.0 % (0/72)	12.5 % (9/72)
SSA	94.1 % (48/51)	80.4 % (41/51)	17.6 % (9/51)	90.2 % (46/51)
TSA	22.2 % (2/9)	11.1 % (1/9)	0.0 % (0/9)	0.0 % (0/9)
CAD	0.0 % (0/51)	0.0 % (0/51)	0.0 % (0/51)	0.0 % (0/51)
MP	50.0 % (3/6)	16.7 % (1/6)	16.7 % (1/6)	16.7 % (1/6)

Table 2 Specific TSA criteria in panel C: kappa value, dependency from pathologist and quality and frequency in HP, SSA, CAD, TSA and MP

TSA criteria	Ectopic crypt formation	Nuclear stratification	Eosinophilic cytoplasm	Nuclear hyperchromasia	Serration>20 %
κ values	0.25	0.82	0.06	0.71	0.49
Pathologist dependency	Yes	No	Yes	No	Yes
Quality dependency	No	Yes	No	Yes	Yes
HP	0.0 % (0/72)	2.8 % (2/72)	0.0 % (0/72)	2.8 % (2/72)	100.0 % (72/72)
SSA	0.0 % (0/51)	0.0 % (0/51)	0.0 % (0/51)	0.0 % (0/51)	100.0 % (51/51)
TSA	66.7 % (6/9)	88.9 % (8/9)	66.7 % (6/9)	88.9 % (8/9)	100.0 % (9/9)
CAD	0.0 % (0/51)	100.0 % (51/51)	0.0 % (0/51)	100.0 % (51/51)	0.0 %(0/51)
MP	16.7 % (1/6)	100.0 % (6/6)	33.3 % (2/6)	100.0 % (6/6)	83.3 % (5/6)

each polyp type as well as inter-observer agreement are listed in Tables 1 and 2.

Diagnostic criteria for SSA correlate with SSA size

As the presence of large serrated polyps increases risk for colorectal cancer [18], we assessed association of single diagnostic criteria with size. SSAs were significantly larger than HPs (mean 0.72 versus 0.58 cm, $p<0.001$). For HPs and SSAs, the criteria hyperserration (0.73 cm, $p<0.001$), T- and L-shaped crypts (0.79 cm, $p<0.001$), inverted crypts (0.96 cm, $p=0.001$) and crypt dilatation (0.69 cm, $p=0.017$) positively correlated with the size of the lesion.

Size of CAD (mean 0.62 cm) and TSA (mean 0.79 cm) was not significantly different in our series ($p=0.16$). In TSA only, ectopic crypt formation correlated positively with size ($p=0.02$).

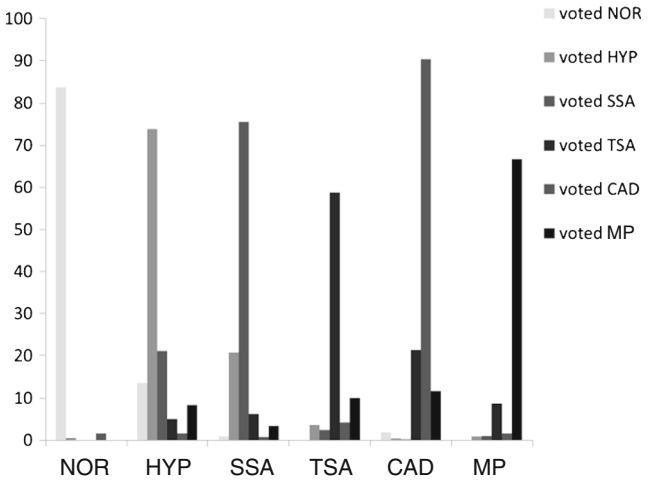

Fig. 2 Aberrant voting for diagnosis in panel A in dependency from the majority's diagnosis (mode). All votes from all observers for one diagnostic category were given a separate colour. These votes for one diagnostic category were set to 100 %. The figure shows their distribution to the final diagnosis. Therefore, the peaks of these judgements reflect the majority's diagnosis as mode value, but note the difficulties in differentiating HYP versus SSA, as well as the diversity of TSA, which were also judged as CAD, HYP or MP

Molecular characteristics correlate with polyp phenotype

Table 3 lists how often *BRAF*, *KRAS*, *NRAS* and *PIK3CA* mutations and *MLH1* promoter methylation occurred in each polyp category. *BRAF* mutations were frequent in HP, SSA and MP. *KRAS* mutations were more frequent in CAD and TSA. The number of *NRAS* and *PIK3CA* mutations was too low to discern a pattern of distribution. *MLH1* methylation occurred in all categories but at a low frequency (mean <5 %).

We then assessed whether or not single criteria for the diagnosis of SSA and TSA correlated with (epi)genetic status. Specific histomorphological criteria for the diagnosis of SSA, like T+L-shaped crypts, basal serration and crypt dilatation, significantly correlated with *BRAF* mutation ($p\leq0.001$). Specific histomorphological criteria for the diagnosis of CAD or TSA, like ectopic crypt formation, nuclear stratification, cytoplasmic eosinophilia and nuclear atypia, correlated significantly with *KRAS* mutation ($p<0.001$). Other proposed histomorphological criteria for the diagnosis of SSA, such as general serration >20 %, did not correlate with specific molecular changes.

We then assessed *BRAF* and *KRAS* mutation status in MP to determine whether they are clonal, which would favour the concept of SSA-D, or composite in containing distinctive components of CAD/SSA, TSA/SSA, CAD/HP or TSA/HP. We hypothesized that coincident *KRAS* and *BRAF* mutations would favour the composite theory, whereas the occurrence of either mutation alone would favour the clonal concept. We found no polyps with coincident *BRAF* and *KRAS* mutations, indicating that MPs are clonal rather than composite which favours the SSA-D concept ($n=6$; χ^2 test, $p<0.001$).

Discussion

Few inter-observer studies have addressed diagnostic reproducibility of serrated polyps of the colorectum. These studies comprised a variable number of observers and generally included preselected cases of serrated polyps [2, 4, 10, 11, 13, 15, 25, 35, 43]. The obtained κ values ranged from 0.14 to

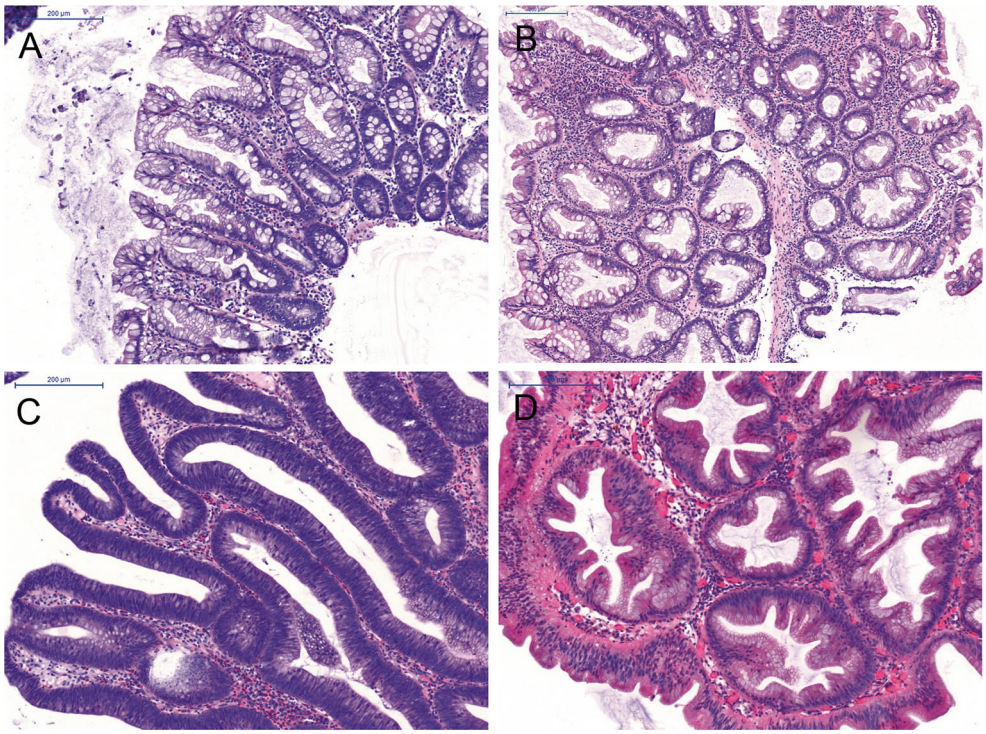

Fig. 3 Samples of polyps with constant diagnosis throughout the panels. Hyperplastic polyp (**a**), sessile serrated adenoma (**b**), classical adenoma (**c**) and traditional serrated adenoma (**d**)

0.65 and were higher for different criteria or subtypes of polyps (up to 0.912) [10].

The aims of our study were to clarify the role of clinical characteristics in the diagnosis, to analyse which single criteria from the German consensus guideline are most discriminative and to correlate these with (epi)genetic characteristics. To this end, we included ten observers who assessed 200 consecutive lesions with the option of six categories (normal, HP, SSA, TSA, MP, CAD). The German consensus guidelines do not contain an unclassifiable category as one other study [10] and used the MP terminology from the previous WHO classification [1], which no longer figures in the recent WHO

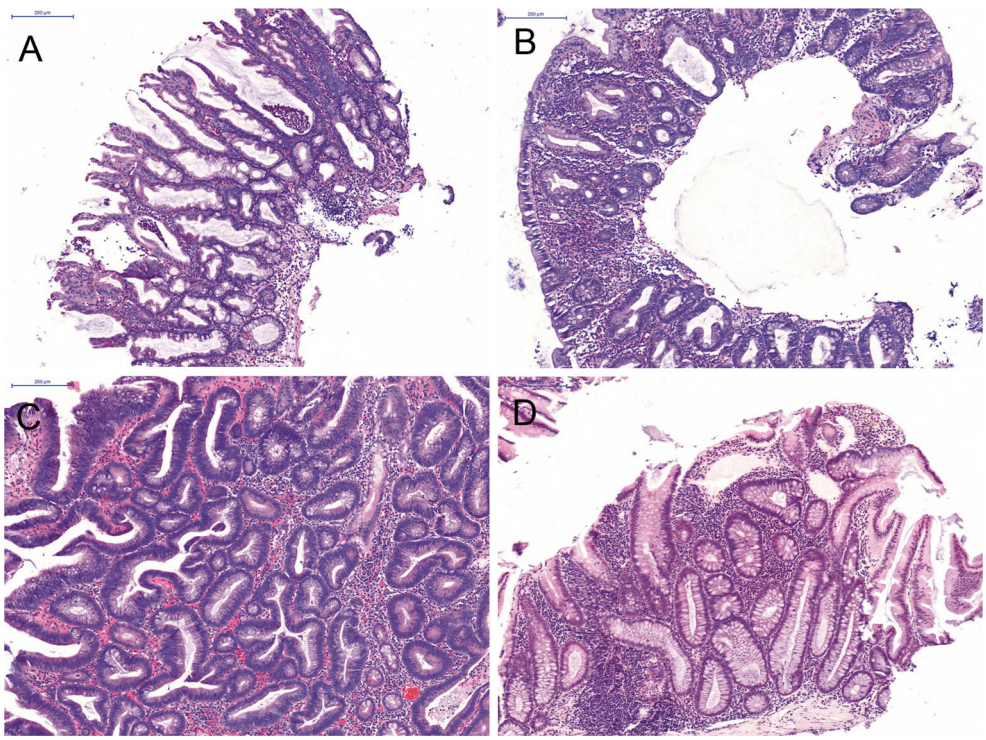

Fig. 4 Samples of variably interpreted lesions. In the *upper part*, lesions are shown with changing diagnosis between HYP and SSA (**a**+**b**). Difficulties arise from present basal serration without additional SSA criteria (**a**) or questionable crypt dilatation (**b**). In the *lower part*, the continuous spectrum between CADs and TSAs is highlighted. Even in clear CADs, some interspersed ectopic crypt formation might be detected (**c**) or even restricted to a specific region (**d**—right side)

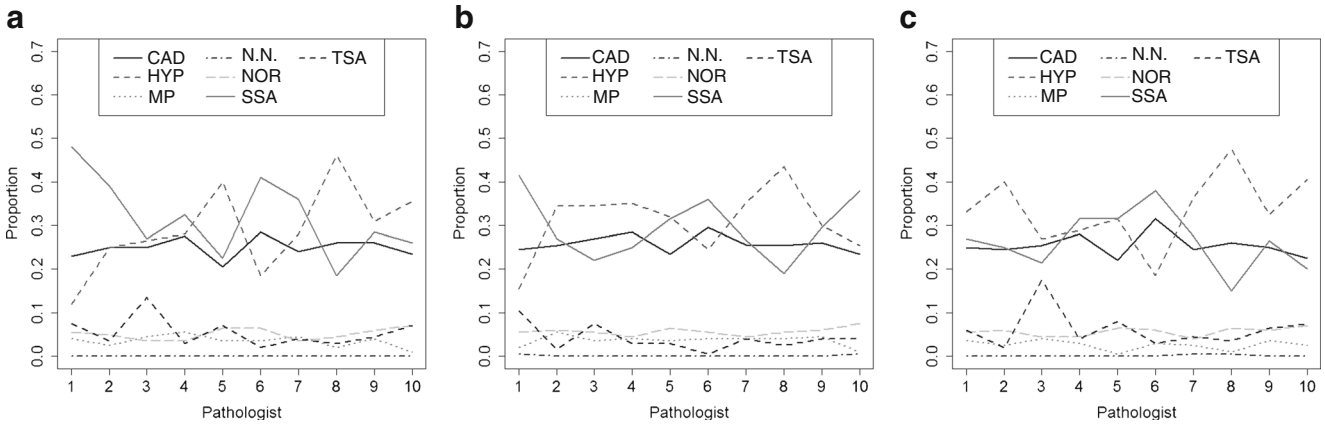

Fig. 5 Diagnostic changes in dependency from singular observers across Panel (**a**), (**b**) and (**c**). Note the narrowing of lines, especially between Panel (**a**) and Panel (**b**), (**c**), but also e.g. the maintenance of moderate (**a–c**, 8) or more aggressive diagnosis of SSA (**a–c**, 6) by some observers despite of the introduced criteria

classification [37] and the proposal from a North American expert panel [34]. Our pre-consensus agreement (panel A) improved from fair to good after we provided clinical data (panel B) and remained at this level after we introduced the German consensus criteria (panel C) [1], which reflects the fact that these criteria must have represented implicit consensus, even before becoming explicit through official publication.

Our findings regarding clinical parameters are in agreement with those from the literature [17, 21, 26, 27]. In particular, location introduces a bias towards the diagnosis of a serrated polyp diagnosis [15]. Our data shed new light on these clinical criteria. As SSA occurs also in the rectum, right-sided should not be regarded as a prerequisite for its diagnosis. Our results support the recent WHO classification and North American expert panel, who both describe SSA as preferably right-sided [1, 34, 37].

Furthermore, the size of the lesion is a matter of importance. In addition to SSA-D, large SSAs are associated with a higher risk of malignant change [26]. Lu et al. retrospectively analysed HP diagnoses made between 1980 and 2001 and found a higher subsequent risk for colorectal cancer when the initial diagnosis was SSA and when the lesions were larger than 5 mm in diameter [28]. Hiraoka et al. found similar results for large serrated polyps (HYP or SSA >10 mm), with an OR of 4.5 for CRC from data of colonoscopic surveillance programmes in Japan [18]. Teriaky et al. investigated 5-year follow-up of patients with SSA and attributed a higher rate of prior or subsequent CRC to SSA (which had a mean diameter of 11 mm) [39]. SSA diagnosis is mainly based on architectural features, the most characteristic of which is the sideways extension of the crypt basis. We found that each single SSA criterion is significantly associated with lesion size. Clinical information on a colorectal polyp should therefore as a rule include the size of the lesion.

The microscopic criteria for SSA with the highest interobserver agreement were (in descending order) T- and L-shaped crypts, basal hyperserration, crypt dilatation and inverted crypts (Table 1). For these, the κ values are comparable to those from other studies [10, 11]. Interestingly, a special subtype of serrated dysplasia which is defined by nuclear features (vesicular type and nucleoli) did not reach satisfactory agreement (κ = 0.07), whereas a strong agreement was reached for classical type adenomatous dysplasia (κ = 0.78). For TSA, the formation of ectopic crypts seems to be the most convincing criterion, whereas the often cited eosinophilic cytoplasm seems to be dependent on individual interpretation (Table 1), in contrast to the published 78 % of agreement obtained on a preselected case series [10]. Nuclear pseudostratification, hyperchromasia and elongation are also features shared with CAD.

Descriptive criteria as such are often broadly accepted among pathologists but when (semi)quantitative, cut-offs may become an issue. This is relevant in the distinction between

Table 3 Tumor genetics of colorectal serrated polyps and frequency of alterations. Quantitative *MLH1* methylation data presented as mean with SEM, *MLH1* hypermethylation was judged at cut-off 17.0 % and is outlined in brackets

Molecular changes	mut *BRAF*	mut *KRAS*	mut *PIK3CA*	mut *NRAS*	*MLH1* methylation
HP	86.1 % (62/72)	9.7 % (7/72)	0.0 % (0/72)	0.0 % (0/72)	3.3±2.4 % (0/72)
SSA	80.4 % (41/51)	7.8 % (4/51)	0.0 % (0/51)	0.0 % (0/51)	3.4±2.9 % (0/51)
TSA	22.2 % (2/9)	66.7 % (7/9)	0.0 % (0/9)	0.0 % (0/9)	2.4±1.1 % (0/9)
CAD	0.0 % (0/51)	19.6 % (10/51)	2.0 % (1/51)	2.0 % (1/51)	6.4±11.0 % (2/51)
MP	83.3 % (5/6)	16.7 % (1/6)	0.0 % (0/6)	0.0 % (0/6)	6.8±5.3 % (0/6)

CAD and TSA, where we relied on >20 % serration as proposed by Bariol et al. [2] and in the distinction between HYP and SSA for which we required (according to the German consensus) two characteristic crypts (not necessarily adjacent). The latter increased the frequency of a diagnosis of HYP. The recent WHO classification even suggests that two or three contiguous crypts should be used as diagnostic criterion [37]. In contrast, the North American expert panel regards one crypt with SSA features as sufficient for diagnosis [34].

Other differences between the German consensus guidelines, the WHO classification and the North American expert panel are the mucin type and the assessment of proliferation in serrated colorectal polyps [34, 37]. Both criteria would rely on immunohistochemistry, e.g. MUC6 or Ki67 staining [32, 40]. In daily practice, polyp diagnosis is based on H&E staining and immunohistochemical staining should be used only in case of diagnostic difficulties, in order to maintain cost-effectiveness.

We found a high prevalence of *BRAF* mutations in HP, SSA and MP, whereas *KRAS* mutations were more frequent in CAD and TSA, as well documented in the literature [6, 24, 41, 44]. As our study was based upon the German consensus guidelines [1], which consider MP to be a mixture of HP or SSA with CAD or TSA components, we maintained MP as a diagnostic category. This concept has been questioned as it does not appropriately address how SSA progress towards carcinoma, which is the reason why the North American expert panel and the new WHO classification both prefer the term SSA-D [34, 37]. Although the number of MP in our study was very low, we interpret the mutual exclusion of *BRAF* and *KRAS* mutations in these lesions as evidence of a clonal nature, favouring the SSA-D approach. It would have been of interest to separately analyse the different components of an MP, through DNA extraction of micro-dissected tissue samples. In our study, DNA extraction was performed on complete polyps prior to the reference diagnosis which precluded differential genetic analysis of defined regions.

BRAF mutation is regarded as an early event in the development of SSA and has been even identified in the SSA-associated microvesicular subtype of HP [5, 31, 33, 38, 45]. Our detailed analysis of single criteria of serrated lesions underlines that basal hyperserration, which might precede other morphological criteria in the progression from HP to SSA, is strongly associated with *BRAF* mutation. *MLH1* methylation is also associated with SSA, but was infrequent in our study maybe because the large majority of our SSA cases were without dysplasia. *MLH1* methylation is indeed considered to be a later event in the serrated pathway of colorectal carcinogenesis [9, 14, 24, 36]. The frequency of *PIK3CA* and *NRAS* mutations in our series was too low to allow any conclusions.

In summary, our study confirms that serrated colorectal polyps should be diagnosed with good inter-observer reproducibility, which might be achieved using the new WHO classification, even though a cut-off problem still needs to be solved [37]. Serrated lesions with true dysplasia should no longer be called MP but SSA-D, unless TSA criteria are fulfilled. Both are rare (we only made 15 diagnoses of TSA or MP in a series of 1,926 consecutive polyps (0.8 %)), but their diagnosis is important in view of their biological behaviour. How molecular classification based on *BRAF*, *KRAS* mutation and *MLH1* methylation might impact on risk of malignant progression needs to be studied in larger prospective series, which has become feasible with the introduction of robust *BRAF* mutation immunohistochemistry [30].

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

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