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ORIGINAL ARTICLE

Combining aneuploidy and dysplasia for colitis' cancer risk assessment outperforms current surveillance efficiency: a meta-analysis

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Abstract
Purpose Cancer risk assessment for ulcerative colitis patients by evaluating histological changes through colonoscopy surveillance is still challenging. Thus, additional parameters of high prognostic impact for the development of colitis-associated carcinoma are necessary. This meta-analysis was conducted to clarify the value of aneuploidy as predictor for individual cancer risk compared with current surveillance parameters.
Methods A systematic web-based search identified studies published in English that addressed the relevance of the ploidy status for individual cancer risk during surveillance in comparison to neoplastic mucosal changes. The resulting data were included into a meta-analysis, and odds ratios (OR) were calculated for aneuploidy or dysplasia or aneuploidy plus dysplasia.
Results Twelve studies addressing the relevance of aneuploidy compared to dyplasia were comprehensively evaluated and further used for meta-analysis. The meta-analysis revealed that aneuploidy (OR 5.31 [95 % CI 2.03, 13.93]) is an equally effective parameter for cancer risk assessment in ulcerative colitis patients as dysplasia (OR 4.93 [1.61, 15.11]). Strikingly, the combined assessment of dysplasia and aneuploidy is superior compared to applying each parameter alone (OR 8.99 [3.08, 26.26]).
Conclusions This meta-analysis reveals that aneuploidy is an equally effective parameter for individual cancer risk assessment in ulcerative colitis as the detection of dysplasia. More important, the combined assessment of dysplasia and aneuploidy outperforms the use of each parameter alone. We suggest image cytometry for ploidy assessment to become an additional feature of consensus criteria to individually assess cancer risk in UC.

Keywords Ulcerative colitis-associated colorectal carcinoma · Nuclear DNA ploidy · Cancer risk assessment · Meta-analysis

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Introduction

In 1925, the first case of rectal carcinoma in ulcerative colitis (UC) was described [1]. Since this observation, an increased incidence of colorectal carcinoma in UC has been reported in numerous scientific publications. Reports on the frequency of UC-associated carcinoma (UCC) vary from 0.2 to 34 % depending on age at diagnosis, extent and duration of UC as well as study design [2]. In a comprehensive meta-analysis by Eaden et al., the cumulative incidence for UCC was stated as 1.6 % after 10 years, 8.3 % after 20 years, and 18.4 % after 30 years of disease duration [3]. However, the actual risk for carcinoma development in UC is still a matter of debate [4–7].

Histopathogenesis of UC-associated colorectal carcinogenesis is widely believed to involve a stepwise progression from

inflamed and hyperplastic epithelium to flat dysplasia and finally adenocarcinoma [8, 9]. In contrast to sporadic colorectal cancer, UCC-related tumor development includes chronic inflammation, injury, dysplasia, and carcinoma, which arise without the formation of a well-defined adenoma [10].

To detect premalignant lesions or UCC at early stages, colonoscopy surveillance is the gold standard as stated in various national and international guidelines [11–15]. However, the sensitivity to detect premalignant lesions via endoscopical screening for dysplasia and cancer is rather low: Rubin et al. demonstrated that only 72 % of all colitis-related premalignant lesions were detected [16]. Furthermore, a subgroup of patients develop UCC after a short disease duration, e.g., Lutgens et al. could demonstrate that 21 % of 89 examined patients developed UCC at a time before cancer surveillance was recommended [17]. Strikingly, we recently found a high rate of preoperatively undetected high-grade intraepithelial neoplasia and carcinoma in UC patients with long-standing inflammation of the colon in a cohort of patients undergoing proctocolectomy [18].

At present, biopsies are analyzed for neoplastic changes of the intestinal mucosa (dysplasia) in order to assess the individual cancer risk. The severity of dysplasia usually is classified according to Riddell et al. [8]. However, the efficacy of surveillance programs based on histopathologic evaluation of dysplasia has been questioned for several reasons. Notably, the occurrence of dysplasia is not mandatory before an UCC develops [19–21]. Dysplasia is absent in 20–30 % of colectomy specimens containing cancer in UC [22]. Moreover, cancer risk assessment based on dysplasia as a marker is hampered by numerous methodical challenges. Many early lesions do not produce endoscopically recognizable abnormalities [23]. Due to the large area of the colon and a patchy distribution of dysplasia, sampling errors by the endoscopist are likely to occur. According to international guidelines, at least four biopsies per every 10 cm should be taken around the colon plus biopsies of macroscopically dysplastic lesions [24]. Nevertheless, a typical biopsy represents less than 0.05 % of the total colonic surface, and the number of biopsies taken by endoscopists in routine practice is often less [25, 26]. In addition to difficulties in sample collection, histopathological evaluation of dysplastic lesions in the inflamed colon mucosa is highly subjective. Classification of lesions according to Riddell et al. [8] is hampered by interindividual and intraindividual variation [19, 27–32]. Therefore, additional reliable parameters of high prognostic impact in individual risk assessment for development of UCC are necessary.

In 1984, Hammarberg et al. reported changes of the nuclear DNA content in colorectal biopsies, followed by several other publications addressing the possible use of ploidy analysis for the assessment of malignancy development [33]. Although an increased incidence of aneuploidy in correlation to an advancing degree of dysplastic mucosal changes has been observed [34–37], discordant occurrence of dysplasia and aneuploidy has been reported [38–40]. Particularly, aneuploidy has been shown to precede dysplasia by 1–2.5 years [23]; thus, the theory of a stepwise cancer genesis with the occurrence of genetic instability at early stages of tumor development resulting in aneuploid cells that transform via dysplasia toward malignancy has been evolved. Additionally, aneuploidy has been found frequently in non-malignant mucosa adjacent to UCCs and seems to be irrespective of dysplasia [41].

The value of ploidy measurements for the prediction of esophageal, gastric, and colorectal tumors was reviewed some years ago by Grabsch et al. [42]. They found aneuploid cell populations in biopsies from UC patients to be prevalent in 6–71.4 % of cases and an increase of aneuploid lesions with the extent of the disease as well as the disease duration [43, 44]. However, most of the chosen studies lack the consideration of individual UC patients progressing to UCC in comparison with ploidy development over time, although in general, the samples descended from surveillance programs. The potential value of ploidy assessment in cancer risk assessment during surveillance is not even considered in most international guidelines [11–15].

Thus, this meta-analysis focuses on the value of aneuploidy for individual cancer risk assessment compared with the actual gold standard of evaluating neoplastic mucosal changes in UC.

Material and methods

Search and extraction process

In January 2016, the PubMed database was searched in order to identify relevant studies without any restrictions in terms of the year of publication based on the following terms: "ulcerative colitis + cancer prognosis + ploidy," "ulcerative colitis + risk assessment + ploidy," "ulcerative colitis + surveillance + ploidy," "ulcerative colitis + cancer prognosis + cytometry," "ulcerative colitis + risk assessment + cytometry," "ulcerative colitis + surveillance + cytometry," and "ulcerative colitis + aneuploidy." Each search was limited to studies on humans published in English. Studies on patients after colectomy and studies on inflammatory bowel diseases other than UC were excluded. There were 173 studies matching these criteria. Among these, surveillance studies considering the ploidy status as well as the actual risk assessment by rating neoplastic mucosal changes for the individual cancer risk during disease development were selected. In total, 12 studies fulfilled the requirements and were included (Table 1) [23, 38, 44–53]. Three of these studies were retrospective; the others were follow-up or prospective studies. In

Table 1 Baseline characteristics of surveillance studies included

Author	Year	Study type	UC patients (*n*)	Preselection criteria	Surveillance			Ploidy analysis					
					Interval (years)	Biopsy sites (*n*)	Period (years)	Method	Sample preparation	Stain	Control	Nuclei per sample	Definition of ploidy status
Choi et al.	2015	R	29	Indefinite for dysplasia	ns	ns	0.1–1.8	FC	Fresh	ns	ns	ns	A
Sjoqvist et al.	2004	P	13	LGD/aneuploidy	0.5	9	2	FC	Fresh	DAPI	ns	ns	B
Habermann et al.	2001	R	24	UCC, duration of disease, extend of inflammation, dysplasia	ns	8	≤13	IC	Paraffin embedded	FG	Internal staining controls	100	C
Holzmann et al.	2001	P	63	Dysplasia, aneuploidy, or extent and duration of UC	1	6	0.3–3.3	FC	Frozen	PI	ns	10,000	D
Lindberg et al.	1999	P	147	All UC patients from 65,000 inhabitants	2/1[a]	6	13	FC	Frozen	PI	ns	ns	D
Karlen et al.	1998	P	12	Dysplasia or aneuploidy	2/1[b]	10	2–13	FC	Fresh	EB	Lymph	1000–50,000	E
Befrits et al.	1994	F	63	Long-standing UC	10 (1–2)[c]	10	10	FC	Fresh	EB	Lymph	ns	F
Rubin et al.	1992	P	25	High-risk patients without dysplasia or cancer	0.5–2.5	~40	>2	FC	Frozen	ns	ns	ns	A
Lofberg et al.	1992	P	59	Long-standing, total colitis	2/1[b]	10	2–8	FC	Fresh	EB	Lymph	ns	G
Rutegard et al.	1989	R	23	Dysplasia	ns	6	10	FC	Paraffin embed-ded	PI	ns	3000–10,000	D
Rutegard et al.	1988	P	73	All UC patients from 65,000 inhabitants	2/1[d]	6	3	FC	Fresh or frozen	PI	ns	ns	D
Lofberg et al.	1987	P	53	UC with 10 years duration	Aperiodic	10	3.5	FC	Fresh	EB	Lymph	1000–50,000	E

Patient numbers only include UC patients who were observed and analyzed in detail over time. Definition of ploidy status: aneuploid: G0/G1 cells with a discrete peak modally separated from the diploid population with a DNA content <1.8 or >2.2 in more than 5 % of nuclei (*A*); diploid: histograms with single peak, aneuploid: additional peak and a corresponding G2 + M peak (*B*); classification according to Auer (four histogram types); aneuploid: histograms showing increased (>5 %) and/or distinctly scattered DNA values exceeding the tetraploid region (>4.5c) (*C*); aneuploid: histograms with more than one peak (*D*); DNA value of G1/G0 cells in relation to control cells. Peaks with values above 2.2c are considered as aneuploid (*E*); aneuploid: DNA indices deviating more than 10 % from 1.0 (*F*); diploid: G1/G0 peak at 2.0c and a corresponding G2 + M peak at 4.0c deviating less than 10 % from the standard lymphocytes; aneuploid: additional distinct G1/G0 peaks ≥2.2c and 4.0c exceeding the normal G2 + M peak more than 3 SD if also a corresponding 8.0c peak was identified (*G*)

P prospective study, *R* retrospective study, *F* follow-up study, *LGD* low-grade dysplasia, *lymph* lymphocytes, *FG* Feulgen, *PI* propidium iodine, *EB* ethidium bromide

[a] After 6–10-year disease duration anually or bianually colonoscopy

[b] 8–20-year disease duration colonoscopy every second year, >20-year disease duration anually colonoscopy

[c] Ploidy measurement after 10 years, clinical follow-up with colonoscopy for histologic evaluation every year or second year

[d] >10-year disease duration colonoscopy every second year, rigid sigmoidoscopy in year between, >20-year anually colonoscopy

Table 2 Studies including unselected patients

Author	Year	Department	Surveillance		Patients	Patients with diagnosis of						Incidence of aneuploidy			
						Cancer		Aneuploidy		Dysplasia		Related to dysplasia (*n*)			
			Period (years)	Years	*n*	*n*	%	*n*	%	*n*	%	Alone	Before	Simult	After
Lindberg et al.	1999	A	13	1984–1997	147	5	3.4	20	13.6	35	23.8	6	4	3	7
Befrits et al.	1994	B	10	1979–1994	36	–[a]	–[a]	3[a]	8.3[a]	5[a]	13.9[a]	–[a]	3[a]	–[a]	–[a]
Rubin et al.	1992	C	>2	ns–1992	25	–	–	6	24.0	10	40.0	–	5	1	–
Lofberg et al.	1992	B	2–8	1982–1990	59	–	–	15	25.3	14	23.7	2	6	6	1
Rutegard et al.	1988	A	3	1984–1987	73	1	1.4	6	8.2	9	12.3	2	2	–	2
Lofberg et al.	1987	B	3.5	1982–1985	53	1	1.9	5	9.4	12	22.6	1	1	3	–
				Total	393	7	1.8	55	14.0	85	21.6	11	21	13	10

Only patients with continued surveillance are considered. Samples classified as "indefinite for dysplasia" were uniformly re-classified as positive. Departments: Departments of Surgery, Örnsköldsvik Hospital, and the Departments of Pathology and Clinical Cytology, University Hospital, Umeå, Sweden (*A*); Unit of Gastroenterology, Department of Medicine, Huddinge University Hospital, Department of Medical Radiobiology, Karolinska Institutet, Department of Pathology and Medical Department II, South Hospital, Stockholm, Sweden (*B*); Division of Gastroenterology, Department of Medicine, and Department of Pathology, University of Washington, Seattle, Washington; Group Health of Puget Sound, Seattle, Washington; and Department of Pathology, University of Tennessee-Baptist Memorial Hospital, Memphis, Tennessee (*C*)

[a] At 10-year follow-up time point

six included studies, patients were preselected for certain specific phenotypes including the presence of dysplasia and/or aneuploidy (Table 3). The remaining six studies were carried out on patients chosen for having UC for a certain duration or extent (Table 2). To allow a simplified comparison of the different study designs, the respective surveillance periods, the intervals of colonoscopy as well as the numbers of biopsy sites and the analysis methods with regard to sample preparation, staining, and evaluation criteria are listed in tabular form (Table 1). The histopathological rating of the biopsies was performed according to Riddell et al. [8] in all included studies, but there were variations in the assessment of the ploidy status with regard to method (flow cytometry (FC) or image cytometry (IC)), sample preparation (fresh, frozen, paraffin embedded) as well as staining and evaluation procedures (Table 1).

Meta-analysis

In order to address whether aneuploidy, dysplasia, or a combination of aneuploidy and dysplasia is associated with the occurrence of an UCC, the frequencies of UCCs, aneuploidy, and dysplasia of each study were summarized and a meta-analysis was conducted on these data. For each study, odds ratios (OR) and appropriate 95 % confidence intervals were calculated. The ORs were combined by assuming random effect models. A test for heterogeneity as well as for publication bias was performed. A p value <0.05 was considered significant. The results were illustrated in forest plots (Figs. 1, 2, and 3). Statistical analysis was performed by using the software R version 2.12.2 (package metafor).

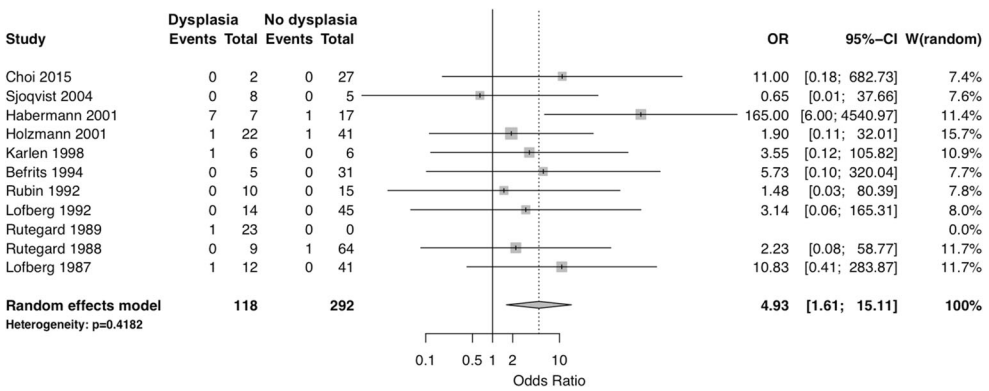

Fig. 1 Forest plot of the OR for detection of dysplasia and UCC occurrence. Studies are listed in order of their year of publication. The 95 % CI is shown for each study (*horizontal line*). The size of the square symbol is proportional to the weight of the study in the pooled estimate by using a random effects model. The *diamond* and the *broken line* represent the overall estimate including all studies and the according 95 % CI, respectively

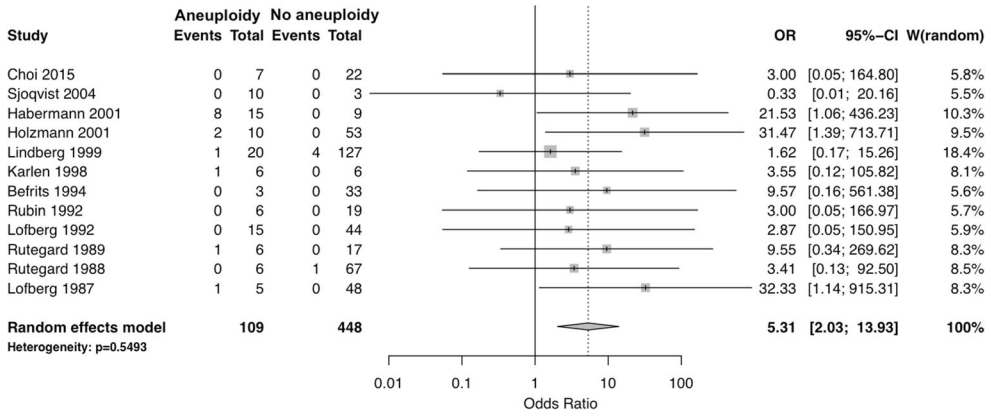

Fig. 2 Forest plot of the OR for detection of aneuploidy and UCC occurrence. Studies are listed in order of their year of publication. The 95 % CI is shown for each study (*horizontal line*). The size of the square symbol is proportional to the weight of the study in the pooled estimate by using a random effects model. The *diamond* and the *broken line* represent the overall estimate including all studies and the according 95 % CI, respectively

Results

Association of DNA aneuploidy and dysplasia in surveillance colonoscopy

Numerous studies concerning aneuploidy in UC have been published. However, only a small number of these have addressed the relevance of the ploidy status regarding the individual cancer risk during surveillance in comparison to the risk assessment by evaluating neoplastic mucosal changes (Table 1).

The first study was performed in 1987 by Lofberg et al. who focused on the correlation of aneuploidy with histological dysplasia during surveillance colonoscopy in UC patients [52]. The prospective study was comprised of 53 patients, and aneuploidy was detected in five of those patients (Table 2). There was aneuploidy but no dysplasia in one patient. Four of the patients also had dysplasia in the aneuploid mucosa. Aneuploidy was detectable in one of those patients before dysplasia was macroscopically diagnosable. In one patient, low-grade dysplasia evolved into high-grade dysplasia and adenocarcinoma was found after colectomy where aneuploidy had been detected before. In general, aneuploidy was detected multifocally but not necessarily in the same location as dysplasia. Additionally, eight patients had dysplastic lesions without detectable aneuploidy. Three of these patients had low-grade dysplasia, and five were indefinite for dysplasia.

In 1988, Rutegard et al. published a 3-year prospective study on 73 UC patients [51]. DNA aneuploidy was found in six patients, and there was aneuploidy but no dysplasia in two of these patients. In two patients, aneuploidy was found before detecting lesions classified as indefinite for dysplasia. No spatial relation between aneuploidy and dysplasia was observed. There were five patients without detectable aneuploidy presenting one carcinoma, one high-grade dysplasia, and three low-grade dysplasias.

The same group published a retrospective study on 23 patients preselected for dysplasia and indefinite mucosal changes [50] (Table 3). Eleven patients were indefinite for dysplasia; nine had low-grade dysplasia, and three had high-grade dysplasia.

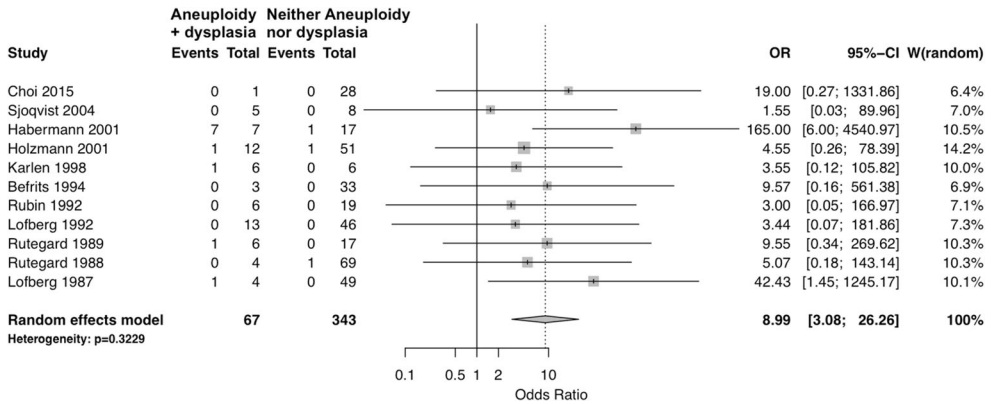

Fig. 3 Forest plot of the OR for detection of dysplasia plus aneuploidy and UCC occurrence. Studies are listed in order of their year of publication. The 95 % CI is shown for each study (*horizontal line*). The size of the square symbol is proportional to the weight of the study in the pooled estimate by using a random effects model. The *diamond* and the *broken line* represent the overall estimate including all studies and the according 95 % CI, respectively

Table 3 Studies including preselected patients.

Author	Year	Department	Surveillance		Selection criterion	UC patients (*n*)	Patients with diagnosis of						Aim of study
							Cancer		Aneuploidy		Dysplasia		
			Period (years)	Years			*n*	%	*n*	%	*n*	%	
Choi et al.	2015	A	0.1–1.8	2003–2013	Indefinite for dysplasia	29	0	0	7	24.1	2	6.9	Outcome of indefinite for dysplasia
Sjoquist et al.	2004	B	2	ns	Aneuploidy and/or low-grade dysplasia	13	0[a]	0.0[a]	10[b]	76.9[b]	8[b]	61.6[b]	Evaluation of ursodeoxycholic acid as therapeutic agent
Habermann et al.	2001	C	≤13	1986–1999	UCC, duration of disease, extent of inflammation, dysplasia	24	8[b]	33.3[b]	15	62.5	7	29.2	CAC marker validation (ploidy, laminin-5 gamma 2, cyclin A)
Holzmann et al.	2001	D	0.3–3.3	ns	Dysplasia, aneuploidy or extent and duration of UC	63	2	3.2	16[b]	25.4[b]	14[b]	22.2[b]	Correlation of aneuploidy and clinical characteristics
Karlen et al.	1998	B	2–13	1982–ns	Colectomy because of dysplasia or medical intractability	12	1	8.3	6[b]	50[b]	6[b]	50[b]	Sialyl-Tn as cancer risk marker in relation to ploidy and dysplasia
Rutegard et al.	1989	E	10	1977–1987	Dysplasia	23	1	4.3	6	26.1	23[b]	100[b]	Association between dysplasia, aneuploidy, and cancer development

Department: Department of Pathology, Rodger C. Haggitt Gastrointestinal and Hepatic Pathology Service, University of Washington, Seattle, Washington, USA (*A*); Unit of Gastroenterology, Department of Medicine, Huddinge University Hospital, Department of Medical Radiobiology, Karolinska Institutet, Department of Pathology and Medical Department II, South Hospital, Stockholm, Sweden (*B*); Department of Surgery and Unit of Gastroenterology, Department of Medicine I; University Hospital Schleswig-Holstein, Campus Lübeck, Lübeck, Germany (*C*); Department of Internal Medicine I, Eberhard-Karls-University Tübingen, Tübingen, Germany, and the Institute of Pathology, Municipial Hospital, Aschaffenburg, Germany (*D*); Department of Surgery, Örnsköldsvik Hospital, and the Departments of Pathology and Clinical Cytology, University Hospital, Umeå, Sweden (*E*)

[a] One of the patients underwent surgery and was found to have cancer before setup of the study

[b] Patients preselected for aneuploidy/dysplasia/carcinoma

Aneuploidy was found in six of these patients. All of the patients with high-grade dysplasia had aneuploidy, and in one of these patients, aneuploidy preceded two diploid carcinomas.

Lofberg et al. followed up 59 patients with long-standing, total UC in a prospective surveillance program for 8 years [38]. Fifteen of these patients had aneuploid biopsies at least once (Table 2). Among these patients, aneuploidy was detected before development of definite dysplasia in six patients, simultaneously with development of dysplasia in another six patients, and after the development of dysplasia in one patient. The remaining two patients with a repeated finding of aneuploidy had no concomitant dysplasia. Remarkably, definite dysplasia developed in all six patients in which aneuploidy had been found before dysplasia after an average time of 2.7 years. During the follow-up period, aneuploidy spread throughout the locality with increasing duration of the disease but persisted in the same part of the colon.

Aneuploidy as a predictor of progression to dysplasia

In 1992, Rubin et al. published a prospective study on high-risk patients without dysplasia or cancer, who were identified by a prevalence study on 101 UC patients [23]. There was a significant trend for an increase of aneuploid lesions concordant with histological progression from indefinite for dysplasia to dysplasia to carcinoma in the prevalence study.

Out of the 101 UC patients, 25 high-risk patients (disease not confined to rectum and sigmoid, disease duration >8 years, or both) were prospectively examined by colonoscopy for at least 2 years. Six of the 25 patients progressed to dysplasia, and 5 of these 6 patients had aneuploidy preceding the finding of dysplasia by 1–2.5 years. The sixth patient had concurrent development of aneuploidy and dysplasia. Nineteen patients did not show aneuploidy at any time of follow-up. All of them had initially been negative for dysplasia; however, on termination of the follow-up, 15 patients were negative and 4 were indefinite for dysplasia. Rubin et al. concluded aneuploidy to be a significant predictor of progression to dysplasia and could show that considerably less biopsies are needed to detect aneuploidy compared with the histological finding of cancer. Ploidy assessment on up to 30 biopsies was needed to detect aneuploidy with 95 % confidence, whereas up to 64 biospies were needed to detect the highest degree of dysplasia with 95 % confidence. Rubin et al. concluded DNA ploidy measurement to be useful to identify aneuploidy in the absence of dysplasia as an indicator of an increased risk for development of dysplasia requiring a more frequent follow-up of the patients [23].

Aneuploidy measurement does replace detection of dysplasia

Befrits et al. published a follow-up study on 36 UC patients in 1994 [49]. The authors initially assessed DNA ploidy and histopathology, then performed annual colonoscopies for 10 years, and repeated the ploidy measurement after 10 years. Initially, 63 UC patients were included to the study, but 27 patients dropped out before the 10-year endpoint. One of those 27 patients received colectomy because of simultaneous finding of low-grade dysplasia and aneuploidy, and the colectomy specimen showed an adenocarcinoma in the same dysplastic/aneuploid area. None of the 36 patients who completed the 10-year follow-up had dysplasia on the first examination. Aneuploidy was initially detected in six of these patients. After 10 years, all 30 initially diploid patients still had no detectable aneuploidy, but low-grade dysplasia was found in 2 patients. Of the initial six aneuploid patients, neither aneuploidy nor dysplasia was found in two patients, one patient was reclassified as diploid, and three patients presented again with aneuploidy and in addition now presented also low-grade dysplasia. In accordance with Lofberg et al. [38], the authors suggest the simultaneous finding of aneuploidy and low-grade dysplasia to be an indication for surgical treatment since they also found one unexpected adenocarcinoma after colectomy.

Karlen et al. prospectively evaluated the mucin-associated sialyl-Tn (STn) antigen as a marker in cancer risk assessment in relation to dysplasia in 1998 [48]. The authors performed a matched case-control study on UC patients who underwent colectomy either for dysplasia (six cases) or for medical reasons (six controls). The control patients by definition showed neither dysplasia nor aneuploidy nor cancer during a surveillance period of 2 to 13 years. This was in contrast to the matched cases: Two cases showed aneuploidy before the finding of dysplasia, and four cases presented aneuploidy and dysplasia simultaneously. In one case, multiple high-grade and low-grade dysplasia as well as widespread aneuploidy was found during surveillance colonoscopies and the colectomy specimen showed a carcinoma. In another case of widespread aneuploidy and repeated findings of indefinite and low-grade dysplasia, the colectomy specimen confirmed indefinite dysplasia only.

Aneuploidy as a marker for development of dysplasia is highly specific

In 1999, Lindberg et al. published a surveillance study on 147 patients with long-standing UC followed for 13 years in intervals of 1 to 2 years [47]. Aneuploidy was found in 20 patients: Six patients had aneuploidy without dysplasia, and in three patients, aneuploidy and dysplasia were detected simultaneously. In four patients, aneuploidy preceded the finding of dysplasia, while in seven patients, dysplastic changes preceded the finding of aneuploidy. Additionally, there were 21 patients without aneuploidy but dysplasia. Eight of these patients were indefinite for dysplasia, seven had low-grade dysplasia, two had a dysplasia-associated lesion/mass (DALM), and four patients had a carcinoma. Three of these carcinomas were

diploid, whereas one was aneuploid. Lindberg et al. calculated the sensitivity of aneuploidy to indicate the development of dysplasia (low-grade dysplasia or higher) to be 0.5 and the specificity to be 0.94. Thus, detection of aneuploidy during surveillance is associated with an increased risk to develop severe dysplasia.

A study on 368 UC patients by Holzmann et al. focused on the prevalence of aneuploidy dependent on the extent of UC and the correlation of aneuploidy with clinical characteristics [44]. Aneuploidy was found in 32 of the 368 patients, and its frequency increased with disease extent and duration. The frequency of aneuploidy increased from 2.9 % in biopsy specimens without dysplasia to 39.6 % in biopsy specimens classified as indefinite for dysplasia and 35.7 % in biopsy specimens classified as low-grade dysplasia up to 80 % in high-grade dysplasia. All carcinomas were aneuploid.

Moreover, Holzmann et al. conducted a surveillance study on 63 UC patients, which were preselected for aneuploidy (10 patients), dysplasia (8 patients), dysplasia and aneuploidy (6 patients) as well as extent and duration of UC (39 patients) [44]. Five out of the ten patients preselected for aneuploidy developed dysplasia and two a carcinoma during follow-up. Aneuploidy was widely distributed throughout the colon.

In a retrospective study on 24 patients, Habermann et al. focused on independent cellular markers including aneuploidy as a predictor for malignant transformation in UC [46]. Patients were divided into two groups: Group A comprised eight patients who underwent surgery for UCC, and group B comprised 16 patients without clinical or morphological signs of malignancy. In group A, aneuploidy was detectable on average 7.8 years before the UCC diagnosis throughout the entire colon and rectum unrelated to dysplasia. In one patient, there was no detection of dysplasia prior to cancer diagnosis. All eight UCCs were aneuploid. Remarkably, there was one UCC patient without any detection of dysplasia prior to cancer diagnosis. In contrast, DNA aneuploidy was found in only seven of 16 patients in group B. Among these seven patients, aneuploidy was found in two patients at the beginning of the observation period and could not be detected in subsequent biopsies. The remaining five patients were found to be aneuploid at the end of the observation period.

Sjoqvist et al. conducted a double-blind, controlled pilot trial in which the potential of the preventing or reverting effect of ursodeoxycholic acid (UDCA) on patients with long-standing colorectal inflammatory bowel disease with existing premalignant findings was analyzed [45]. Thirteen UC patients with extensive colitis and low-grade dysplasia and/or aneuploidy were included. Seven UC patients were included to the treatment group, while the remaining six received placebos. Six out of seven and four out of six patients in the treatment and placebo group, respectively, showed aneuploidy. Two of the UC patients in the treatment group underwent surgery before inclusion to the study because of DALM with high-grade dysplasia and a carcinoma, respectively. During the study, one UC patient of the placebo group developed dysplasia and underwent colectomy, but there was no carcinoma found in the colectomy specimen.

Choi et al. recently published a retrospective analysis on the correlation of ploidy and the outcome of UC and Crohn's disease patients [53]. In a total of 29 UC patients who were diagnosed with a lesion indefinite for dysplasia, DNA ploidy was measured by flow cytometry and patients were followed up for 1 to 96 months. Aneuploidy or dysplasia was found in seven and two of those patients, respectively, and aneuploidy and dysplasia were found in one patient. None of the included UC patients developed an UCC during follow-up. However, in general, Choi et al. found a strong correlation between the finding of aneuploidy in patients who were initially classified as indefinite for dysplasia on histological examination and subsequent detection of neoplastic lesions [53].

Meta-analysis on prognostic impact of either aneuploidy, dysplasia, or aneuploidy plus dysplasia for UCC development

To evaluate the significance of aneuploidy, dysplasia, or a combination of aneuploidy and dysplasia as prognostic markers for the development of an UCC, the frequencies of UCCs, aneuploidy, and dysplasia of each study were summarized (Table 4) and meta-analyses of these data were conducted subsequently (Figs. 1, 2, and 3). The test for heterogeneity was not rejected for all three analyses. Neither funnel plots (data not shown) nor rank correlation tests revealed evidence for a potential publication bias. ORs were calculated for all studies for aneuploidy, dysplasia, and the combination of aneuploidy and dysplasia, with the exception of dysplasia in Rutegard et al. (1989) as there were no patients without dysplasia in the group of patients without detection of an UCC [50].

Almost all point estimations of the OR give evidence that there is a significantly higher chance to develop an UCC if aneuploidy or dysplasia or aneuploidy plus dysplasia has been detected. Based on all studies included, for aneuploidy, the overall OR is 5.3 [95 % CI 2.03, 13.93], for dysplasia 4.93 [1.61, 15.11], and for aneuploidy plus dysplasia 8.99 [3.08, 26.26].

Hence, aneuploidy is an equally effective parameter for UCC risk assessment as dysplasia. Strikingly, the combined assessment of dysplasia and aneuploidy is superior compared to applying each parameter alone. Interestingly, ploidy analysis by image cytometry compared to flow cytometry was a more accurate predictor.

Discussion

At present, dysplasia is used as a marker of impending malignant transformation in surveillance colonoscopy in UC.

Table 4 Frequency of detection of aneuploidy or dysplasia as well as aneuploidy plus dysplasia as surrogate parameters for UCC development

Author	Year	UC patients (*n*)	Aneuploidy		Dysplasia		Aneuploidy and dysplasia		UCC total (*n*)	Diagnosis of aneuploidy before dyplasia (years)
			Patients (*n*)	UCC (*n*)	Patients (*n*)	UCC (*n*)	Patients (*n*)	UCC (*n*)		
Choi et al.	2015	29	7	0	2	0	1	0	0	–
Sjoqvist et al.	2004	13	10	0	8	0	5	0	0	–
Habermann et al.	2001	24	15	8	7	7	7	7	8	7.8 (Average)
Holzmann et al.	2001	63	10	2	22	1	12	1	2	1
Lindberg et al.	1999	147	20	1	35	ns	14	ns	5	–9[a]
Karlen et al.	1998	12	6	1	6	1	6	1	1	0[b]
Befrits et al.	1994	36	3	0	5	0	3	0	0	–
Rubin et al.	1992	25	6	0	10	0	6	0	0	–
Lofberg et al.	1992	59	15	0	14	0	13	0	0	–
Rutegard et al.	1989	23	6	1	23	1	6	1	1	0[b]
Rutegard et al.	1988	73	6	0	9	0	4	0	1	–
Lofberg et al.	1987	53	5	1	12	1	4	1	1	0[b]

ns not specified

[a] Low-grade dysplasia was diagnosed 9 years before aneuploidy

[b] Aneuploidy and dysplasia were found simultaneously on first colonoscopy

However, the efficacy of surveillance programs based on histopathologic evaluation of dysplasia has been questioned for several reasons, and additional reliable parameters of high prognostic impact in individual risk assessment for development of UCC are necessary.

Nuclear DNA assessment is considered to be a promising marker in risk assessment during surveillance. Measurement of aneuploidy in comparison to dysplasia is in fact more objective and less sensitive to assessment error, one of the major obstacles associated with dysplasia [29]. Overall, the mean age at first detection of aneuploidy was 40.2 ± 11.8 years, and the mean duration of the disease was 20.0 ± 8.6 years.

Dysplastic lesions were found considerably more frequently than aneuploidy, considering all studies on unselected UC patients (Table 2). However, numerous dysplastic lesions were reactive, inflammatory changes of the colonic mucosa, which were considered to be indefinite for dysplasia. Aneuploidy was absent in the majority of these cases [23, 47, 51, 52]. In the collective of Lofberg et al., five patients underwent surgery because of detection of dysplasia and aneuploidy [38]. Aneuploidy was more reproducible in the surgical specimens than dysplasia, and in one case, severe inflammatory changes made histopathologic evaluation impossible, but aneuploidy was detectable reliably. Thus, DNA assessment is a useful tool in discrimination of inflammatory changes and dysplasia.

In numerous cases, aneuploidy tended to be found more widespread in the course of the disease, but occasionally, aneuploidy was detected once but could not be found again in subsequent examinations [38, 46, 47, 49, 50]. In two patients, aneuploidy was intermittently undetectable [48, 52]. This observation raises the question whether changes in DNA content are reversible or aneuploidy has not been detected again due to sampling errors. Indeed, there are several studies indicating that aneuploidy is reversible in the bronchial mucosa in dogs [54, 55].

Aneuploidy as well as dysplasia was found to be patchy over the colon and rectum without any obvious spatial relation to each other, but aneuploidy tended to be found more widespread throughout the entire colon and rectum [38, 44, 46]. Rubin et al. calculated the number of biopsies needed for reliable detection of the highest category of aneuploidy or dysplasia with a confidence of 95 %. At least 56 biopsy specimens are needed for the detection of definite dysplasia, while 30 biopsy specimens are sufficient for detection of the respective grade of aneuploidy [23]. Although at least four biopsies per every 10 cm around the colon for assessment of dysplasia are recommended by international guidelines [24], there was little consent about the number of biopsies needed for nuclear DNA assessment (Table 1). In Rubin et al., samples were taken from four quadrants in intervals of 10 cm [23]. Samples were subdivided into two parts for histopathologic evaluation and ploidy measurement, respectively. We suggest this procedure of sampling to become part of the consensus criteria for clinical routine standard. There were several cases of aneuploidy without detection of dysplasia; thus, progression from aneuploidy to dysplasia could not be demonstrated consistently. However, this phenomenon might be attributable to a lag of long-term follow-up of patients. There is also

evidence to suggest that malignant transformation arises before or without preceding detectable dysplasia [51].

Almost all included studies were based on nuclear DNA assessment by flow cytometry. However, we favor ploidy evaluation by means of image cytometry, since it allows the measurement of single nuclei in combination with (histo-/cyto-)morphological assessment. This enables the investigator to avoid inflammatory cells, to analyze small sample sizes, and to identify small aneuploid subpopulations. Image cytometry was found to be more sensitive for detection of aneuploidy in comparison to flow cytometry [56, 57]. Particularly, DNA assessment by flow cytometry on paraffin-embedded samples may be hampered by several measurement errors [58]. Besides methodological differences in assessment of DNA content, a number of different methods in terms of preparation of the biopsy specimens (fresh, frozen, paraffin embedded) used controls and the definition of aneuploidy was found in the included studies. Since preparation and storage of the biopsy specimens as well as the analysis and interpretation of aneuploidy assessment are crucial, common guidelines in DNA content analysis are essential for adequate evaluation of biopsies.

In conclusion, our meta-analysis revealed that aneuploidy is an equally effective parameter for UCC risk assessment as dysplasia. Strikingly, the combined assessment of dysplasia and aneuploidy is superior compared to applying each parameter alone. Thus, detection of dysplasia and/or aneuploidy will indicate high-risk patients affording timely follow-up. Conversely, patients with normal findings on DNA content and histopathologic evaluation can be examined less frequently. Thus, aneuploidy assessment should become part of consensus guidelines as complementing risk parameter.

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Compliance with ethical standards

Competing interests The authors declare that they have no conflicts of interest.

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