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Lack of Prognostic Value of Flow Cytometric DNA Content Analysis in Colorectal Adenocarcinomas

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A RECENT CONSENSUS review of the clinical utility of DNA flow cytometry in colorectal cancer has clearly indicated that the vast majority of the reports which showed a positive prognostic significance of DNA ploidy used paraffin-embedded archival material [1]. To date, only a few studies on large numbers of patients using fresh/frozen material and with a long follow-up period have been published [1–4]. By investigating, with DNA flow cytometry, fresh/frozen multiple samples from 119 patients after a follow-up ranging from 1 to 96 months and performing a multivariate analysis, we found no correlation between DNA ploidy and survival (Table 1). Anatomic site (rectum versus colon) and Dukes' stage were the only parameters significantly associated with survival.

Some authors have found a higher risk of death among selected subgroups of patients with DNA aneuploid tumours, such as Dukes' B [2, 6] and C stages [6]. When analysing the prognostic role of DNA aneuploidy in the subgroup of Dukes' B and C patients (n = 93), we failed to confirm this result.

In conclusion, DNA ploidy may not be a significantly independent prognostic factor, according to our current methods of analysis and classification. We suggest that one still needs to perform DNA flow cytometry on a larger number of homogeneous cases using fresh/frozen material and following, with greater attention than in the past, the recent consensus on the guidelines for implementation of clinical DNA cytometry [7]. We also think that new analyses should be performed to investigate the role of DNA index subclasses (DNA aneuploidy in the near-diploid and triploid regions, DNA tetraploidy, which is ill-defined, etc.), since there are clear indications that DNA aneuploidy evolves during colorectal tumour progression into higher DNA index values [8], and that such values are associated with specific molecular DNA lesions, such as TP53 gene loss, known to be a late event in colorectal carcinogenesis [9, 10].

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Table 1. Multivariate Cox analysis on 119 colorectal adenocarcinomas

Variable	No. of patients	RR	CI	P
Sex				
Males	66	Ref		
Females	53	0.57	0.32–1.02	0.056
Site				
Rectum	63	Ref		
Colon	56	0.53	0.30–0.96	0.03
Duke's stage* (continuous)		2.55	1.75–3.70	0.0004
DNA diploid	35	Ref		
DNA aneuploid	84	0.80	0.44–1.45	n.s.

RR, relative risk; CI, 95% confidence interval of RR; P, statistical significance P value; n.s., non-significant; Ref, reference group with RR = 1. *Duke's stage: A = 8 patients, B = 55 patients, C = 38 patients, D = 18 patients.

1. Bauer KD, Bagwell CB, Giaretti W, *et al.* Consensus review of the clinical utility of DNA flow cytometry in colorectal cancer. *Cytometry* 1993, **14**, 486–491.
2. Armitage NC, Ballantyne KC, Sheffield JP, Clarke P, Evans DF, Hardcastle JD. A prospective evaluation of the effect of tumor cell DNA content on recurrence in colorectal cancer. *Cancer* 1991, **67**, 2599–2604.
3. Rognum TO, Lund E, Meling GI, Langmark. Near diploid large bowel carcinomas have better five years survival than aneuploid ones. *Cancer* 1991, **68**, 1077–1081.
4. Enker WE, Kimmel M, Cibas ES, Cranor ML, Melamed MR. DNA/RNA content and proliferative fractions of colorectal carcinomas: a five-year prospective study relating flow cytometry to survival. *J Natl Cancer Inst* 1991, **83**, 701–708.
5. Jones DJ, Moore M, Schofield PF. Prognostic significance of DNA ploidy in colorectal cancer: a prospective flow cytometric study. *Br J Surg* 1988, **75**, 28–33.
6. Schutte B, Reynders M, Wiggers T, *et al.* Retrospective analysis of the prognostic significance of DNA content and proliferative activity in large bowel carcinoma. *Cancer Res* 1987, **47**, 329–335.
7. Shankey TV, Rabinovitch PS, Bagwell B, *et al.* Guidelines for implementation of clinical DNA cytometry. *Cytometry* 1993, **14**, 472–477.
8. Giaretti W, Santi L. Tumor progression by DNA flow cytometry in human colorectal cancer. *Int J Cancer* 1990, **45**, 597–603.
9. Offerhaus GJA, De Feyter EP, Cornelisse CJ, *et al.* The relationship of DNA aneuploidy to molecular genetic alterations in colorectal carcinoma. *Gastroenterology* 1992, **102**, 1612–1619.
10. Meling GI, Lothe RA, Borresen AL, *et al.* The TP53 tumor suppressor gene in colorectal carcinomas. II. Relation to DNA ploidy pattern and clinicopathological variables. *Br J Cancer* 1993, **67**, 93–98.

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