# DNA Ploidy and S-Phase Fraction as Prognostic Factors in Surgically Resected Gastric Carcinoma: A 7-Year Prospective Study

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**Abstract.** *Background: DNA ploidy and S-phase fraction (SPF) measured by DNA flow cytometry (FC) have been previously shown to correlate with several clinicopathological variables in several types of tumours. Patients and Methods: DNA FC was performed on multiple frozen tumour samples obtained from 115 patients undergoing curative surgery for gastric cancer (GC). The findings were prospectively tested for correlation with traditional clinicopathological indicators of prognosis. Results: Overall, 20 tumours (17.4%) were diploid, 46 (40.0%) monoclonal and 49 (42.6%) multiclonal. Excluding 4 patients who died within 1 month of surgery, high SPF (>9.6%) was detected in 55 patients (49.6%) and was found to be significantly associated with vascular invasion and multiclonality (p=0.02). An association of borderline statistical significance emerged with macroscopic type (p=0.06) and pN and pM status (p=0.07). Multivariate regression analysis did not show a significant effect of SPF (p=0.11) or DNA ploidy (p=0.28) on 7-year survival. Conclusion: Aneuploidy appears to be a prognostic factor of low penetrance, whereas SPF is a more promising parameter of tumour aggressiveness in patients with GC.*

The last few decades a reduction in incidence and mortality rates of gastric cancer (GC) in both sexes has been observed (1). According to recently published data, 16,454 new cases of GC have been estimated in Italy, with 10,897 deaths (2). The district of Florence continues to be a high risk area for this neoplasm which, in a survey of the Tuscany Cancer Registry over the period 1987-2003, represents GC as the fourth cause of death from cancer after lung, colorectal and prostate carcinomas in men, and as the third cause of death after breast and colorectal cancer in women.

Despite improvements in diagnostic and therapeutic approaches, the prognosis of GC remains unfavourable, with a median 5-year survival rate lower than 25% (1). The depth of wall infiltration and the involvement of regional lymph nodes are well-defined prognostic indicators but do not always predict outcome in individual cases (3). There is, therefore, a strong need to establish parameters referring to cancer biology which can be used together with the traditional factors of tumour aggressiveness so as both to ensure a more accurate prognosis and to enable the rational programming of adjuvant therapies.

The prognostic relevance of DNA ploidy and S-phase fraction (SPF), as measured by flow cytometry (FC), has been under debate for many years; some studies showed a correlation, while others failed to do so (4-8).

The aims of this study were to assess clinicopathological variables, DNA ploidy and SPF in a prospective series of 115 GC subjected to radical surgery, and to evaluate their impact on clinical outcome by univariate and multivariate analyses.

## Patients and Methods

*Patients.* The present study was carried out on 115 cases of GC subjected to curative resection – namely total/subtotal gastrectomy and D1/D2 lymph node dissection with omentectomy – at the Department of Florence University Hospital in the period between February 1995 and October 1999.

All the gastric tumours were confirmed histologically and defined according to the Laurén, Ming and Japanese Research

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Society for Gastric Cancer (JRSGC) classifications for histological type (9-11), the World Health Organisation (WHO) classification for grade of tumour differentiation (12) and the American Joint Committee on Cancer (AJCC) classification for depth of invasion into the gastric wall (pT) and involvement of regional lymph nodes (pN) (13).

Patients were followed-up every 3 months for the first 2 years, at 6-month intervals for the subsequent 2 years and annually thereafter. The follow-up programme included a clinical examination, biochemical tests, abdominal ultrasonography, chest X-ray and endoscopy. Vital status information was sought for all patients by linking up to Municipal Population Offices on December 1, 2006. Information on the vital status at 7 years was available for all patients. To eliminate bias due to postoperative deaths, patients who died within 30 days of surgery were excluded from the survival analysis.

*Tumour samples.* Multiple sampling (up to 4 fragments) was carried out within 15 minutes of surgical resection by the pathologist on lesions having a diameter greater than 1 cm. In each case, normal-appearing, tumour-free mucosa was also obtained as distant as possible from the tumour site and used as a control. Tissue samples were bisected and one half was fixed in formalin and embedded in paraffin for histological diagnosis, while the remaining half was frozen immediately and stored at –80˚C. The adequacy of each specimen was confirmed using frozen tissue sections: only those samples containing more than 80% of neoplastic cells underwent cytometric investigation.

*DNA flow cytometry.* Following mechanical mincing of the tissue fragments, cell suspensions were sieved through 30 μm nylon mesh in order to remove cell clusters. After two washings in phosphate-buffered saline (PBS) (150 mmol/L, pH 7.2), the cells were treated with RNase 0.5 mg/ml (Boehringer Mannheim, Indianapolis, IN, USA) for 30 min at 37˚C and then stained with propidium iodide (PI, 50 μg/ml in sodium citrate 0.1%; Sigma-Aldrich, Milano, Italy) for 12 h at 4˚C. Samples were analysed by means of a Coulter Epics Elite (Coulter Electronics, Hialeah, FL, USA) flow cytometer, equipped with a 488 nm, 15 mW argon ion laser. A minimum of 20,000 nuclei were acquired for each fragment. The DNA ploidy pattern and SPF were determined using the Multicycle 2.53 software program (Phoenix, San Diego, CA, USA) (14-15).

A cell population was considered diploid when the corresponding histogram showed a single symmetrical G0/G1 peak. The populations showing two or more distinct G0/G1 peaks and corresponding G2/M peaks were considered aneuploid. The aneuploid clone deviation was defined by the DNA Index (DI), equivalent to the ratio between the fluorescence channel of the G0/G1 peak of the aneuploid clone and that of the diploid clone. In assessing tumour heterogeneity on the basis of the DNA content, DNA aneuploid cases were further subdivided into monoclonals (with only one aneuploid peak) and multiclonals (with two or more aneuploid peaks with DI differing by at least 10%). In patients with multiple samples, the higher value of SPF was considered. Histograms with coefficients of variation (CV) less than 8% and a percentage of background debris (BD) lower than 20% were taken as adequate for the study.

*Statistical analysis.* Information collected from each patient was retrieved and checked before comparison with vital status information. Simple cross-tabulations (two-tailed Fisher's exact test or Chi-square test for trend, as appropriate) were used to compare different parameters. The Mann-Whitney test was used to compare two groups of cases on one variable.

For the survival analysis, the date of surgery was used as the start of observation. Survival time was calculated from the date of surgery to the date of the last follow-up (December 1, 2006) or of death. The crude probability of survival was estimated using the Kaplan-Meier method and differences between patient groups were assessed by the log-rank test. Survival comparisons were carried out using Cox proportional hazard regression models. Estimated relative risks of dying were expressed as adjusted hazard ratios (HR) and the corresponding 95% confidence intervals (95% CI). First, a multivariate model including age, sex and each variable of interest was used to evaluate the effect of each parameter of interest. Then a regression model with stepwise selection was performed to identify the major death predictors. Finally, a multivariate model including age, sex, pathological stage at diagnosis (pT and pN status), tumour site, Ming classification, SPF and DNA ploidy was used to evaluate the effect of each parameter included in the model. Statistical results were considered significant at a $p$-value $<0.05$. All statistical tests were performed by the SAS software (Version 9.1; SAS Institute Inc., Cary, NC, USA).

## Results

*Flow cytometric analysis.* In the original series of 115 GC cases, sampling was single in 11 cases (9.6%), 2 or 3 specimens were analysed in 91 cases (79.1%) and more than 3 were taken in the remaining 13 cases (11.3%). Out of the 324 tumour samples taken, 79 (24.4%) were diploid, 239 (73.8%) aneuploid and 6 (1.8%) were not adequate for examination. Overall, CV and BD were 4.35% (1.7-7.9%) and 5.0% (0.3-19.3%), respectively.

Regarding the DNA ploidy, 20 cases (17.4%) were diploid, 46 (40.0%) monoclonal and 49 (42.6%) multiclonal. The distribution of cases in relation to the DNA content was compared to the number of samples examined and showed a decreasing frequency of diploid cases (from 27.3 to 7.7%) as the number of samples increased ($p=0.28$, Chi-square for trend). The overall median of the SPF was 9.6% (1.9-35%) which was higher than that of normal mucosa (2.7%; 0.8-21.3%). The SPF median values were 7.6% (1.9-21.6%), 8.0% (3.1-29.0%) and 13.2% (3.4-35.0%) for diploid, monoclonal and multiclonal cases respectively (diploid *vs.* monoclonals: $p=0.13$; diploid *vs.* multiclonals: $p=0.005$; monoclonals *vs.* multiclonals: $p=0.07$, Mann-Whitney test). The median tumour SPF value (9.6%) was employed as the cut-off to separate the cases into high and low SPF subgroups.

*Clinicopathological findings.* Out of the 115 GC patients, 4 (3.5%) died within 1 month of surgery and were excluded from the statistical analysis. Table I gives the distribution of the remaining 111 GC patients according to selected clinicopathological characteristics. The mean age at

Table I. *Distribution of 111 GC cases according to selected clinicopathological variables.*

| Clinicopathological feature | Patients | |
|---|---|---|
| | N | (%) |
| Gender | | |
| Male | 74 | (66.7) |
| Female | 37 | (33.3) |
| Age group (years) | | |
| ≤66 | 56 | (50.5) |
| >66 | 55 | (49.5) |
| Location | | |
| Antrum | 63 | (56.8) |
| Other site | 48 | (43.2) |
| Macroscopic type | | |
| Early | 16 | (14.4) |
| Advanced | 95 | (85.6) |
| Histological grade | | |
| Poorly differentiated | 69 | (62.2) |
| Moderately differentiated | 38 | (34.2) |
| Well-differentiated | 4 | (3.6) |
| Laurén classification | | |
| Diffuse | 32 | (28.8) |
| Other type (intestinal + mixed) | 79 | (71.2) |
| Ming classification | | |
| Infiltrating | 75 | (79.0) |
| Expanding | 20 | (21.0) |
| *Helicobacter pylori* infection | | |
| Absent | 62 | (55.9) |
| Present | 49 | (44.1) |
| pT | | |
| 1-2 | 34 | (30.6) |
| 3-4 | 77 | (69.4) |
| pN | | |
| Negative | 36 | (32.4) |
| Positive | 75 | (67.6) |
| pM | | |
| Negative | 94 | (84.7) |
| Positive | 17 | (15.3) |
| Stage | | |
| I | 27 | (24.3) |
| II | 15 | (13.5) |
| III | 52 | (46.9) |
| IV | 17 | (15.3) |
| Serosal invasion | | |
| Absent | 34 | (30.6) |
| Present | 77 | (69.4) |
| Vascular invasion | | |
| Absent | 47 | (42.3) |
| Present | 64 | (57.7) |
| DNA ploidy | | |
| Diploid | 20 | (18.0) |
| Monoclonal | 43 | (38.7) |
| Multiclonal | 48 | (43.3) |
| S-phase fraction (SPF) | | |
| High (>9.6) | 55 | (49.6) |
| Low (≤9.6) | 56 | (50.4) |
| Vital status at 7 years | | |
| Alive | 44 | (39.6) |
| Dead | 67 | (60.4) |
| Total | 111 | (100.0) |

diagnosis (±SD) was 65.6 years (±11.3) and males comprised 66.7% (74/111) of the study group. Sixty-three tumours (56.8%) were located in the antrum, and most of the cancers were of advanced macroscopic type (85.6%) with an infiltrative growth pattern according to the Ming classification (79.0%). A diffuse type according to the Laurén classification was present in 32 cases (28.8%), and 69 (62.2%) were poorly differentiated. As detailed in Table II, the majority of cases showed an advanced stage (pT3-4: 69.4%; pN+: 67.6%). Forty-four patients (39.6%) were still alive at 7 years from the start of observation (mean time of follow-up: 9.55 years ±1.55; range: 7.15-11.79 years).

*Association of nuclear DNA content and SPF with clinicopathological parameters.* In Table II, the cytometric variables (DNA ploidy and SPF) are compared with clinicopathological characteristics. No significant association with DNA ploidy was found, while SPF values tended to be higher in locally advanced cancers ($p=0.06$, Chi-square) and in cases with lymph node involvement ($p=0.07$, Chi-square) or distant metastasis ($p=0.07$, Chi-square). A significant positive association was found between higher SPF values and vascular invasion ($p=0.02$, Chi-square). SPF values were also higher with increasing cancer cell heterogeneity ($p=0.02$, Chi-square for trend). No association was found between DNA ploidy or SPF and vital status at 7 years.

*Impact on survival.* In our series, overall 7-year survival was 39.6% (30.5-48.6%), with no significant difference in terms of cytometric variables. Although patients having tumours with high SPF tended to have a lower survival rate (34.6% *vs.* 44.6%; $p=0.14$, log rank test) (Figure 1), DNA ploidy did not appear to significantly influence survival (diploid: 45.0%; monoclonal: 41.9%; multiclonal: 35.4%; $p=0.71$, log rank test).

Table III shows the survival analysis at 7 years, estimated by separate Cox regression models adjusted by gender, age and each parameter of interest. All parameters examined, with the exception of DNA ploidy, significantly correlated with survival expectancy ($p<0.05$, Chi-square). SPF also emerged as a significant prognostic factor, indicating an increased risk of death in those cases with higher SPF values (OR=1.79; 95% CI 1.08-2.97; $p=0.025$, Chi-square). However, by means of a Cox proportional hazard regression model with stepwise selection, it was found that factors independently associated with survival were age over 66 years ($p=0.001$, Chi-square), TNM parameters ($p=0.02$, $p=0.026$ and $p=0.001$, respectively; Chi-square) and antrum site ($p=0.04$, Chi-square) (data not shown). Multivariate analysis adjusted for selected parameters (age, gender, pT and pN status, tumour site, Ming classification, cytometric variables) showed a modest effect of SPF on 7-year survival (HR=1.57; 95% CI 0.90-2.76; $p=0.11$, Chi-square) (Table IV).

Table II. *Distribution of 111 GC cases according to clinicopathological features and cytometric variables.*

| | DNA ploidy | | | | SPF | | |
|---|---|---|---|---|---|---|---|
| Characteristic | D | A-mon | A-mul | *P*-value† | Low | High | *P*-value† |
| Gender | | | | | | | |
| Male | 11 (55.0) | 29 (67.4) | 34 (70.8) | | 36 (64.3) | 38 (69.1) | |
| Female | 9 (45.0) | 14 (32.6) | 14 (29.2) | 0.50 | 20 (35.7) | 17 (30.9) | 0.69 |
| Age group | | | | | | | |
| ≤66 | 13 (65.0) | 20 (46.5) | 23 (47.9) | | 24 (42.9) | 32 (58.2) | |
| >66 | 7 (35.0) | 23 (53.5) | 25 (52.1) | 0.35 | 32 (57.1) | 23 (41.8) | 0.13 |
| Location | | | | | | | |
| Antrum | 13 (65.0) | 23 (53.5) | 27 (56.3) | | 32 (57.1) | 31 (56.4) | |
| Other site | 7 (35.0) | 20 (46.5) | 21 (43.7) | 0.69 | 24 (42.9) | 24 (43.6) | 1.0 |
| Macroscopic type | | | | | | | |
| Early | 3 (15.0) | 8 (18.6) | 5 (10.4) | | 12 (21.4) | 4 (7.3) | |
| Advanced | 17 (85.0) | 35 (81.4) | 43 (89.6) | 0.54 | 44 (78.6) | 51 (92.7) | 0.06 |
| Histological grade | | | | | | | |
| Poorly differentiated | 16 (80.0) | 25 (58.1) | 28 (58.3) | | 34 (60.7) | 35 (63.6) | |
| Moderately differentiated | 4 (20.0) | 16 (37.2) | 18 (37.5) | | 19 (33.9) | 19 (34.6) | |
| Well-differentiated | 0 (0) | 2 (4.7) | 2 (4.2) | 0.46 | 3 (5.4) | 1 (1.8) | 0.60 |
| Laurén classification | | | | | | | |
| Diffuse | 9 (45.0) | 11 (25.6) | 12 (25.0) | | 16 (28.6) | 16 (29.1) | |
| Other type | 11 (55.0) | 32 (74.4) | 36 (75.0) | 0.21 | 40 (71.4) | 39 (70.9) | 0.56 |
| Ming classification | | | | | | | |
| Infiltrating | 13 (76.5) | 29 (82.9) | 33 (76.7) | | 37 (84.1) | 38 (74.5) | |
| Expanding | 4 (23.5) | 6 (17.1) | 10 (23.3) | 0.77 | 7 (15.9) | 13 (25.5) | 0.19 |
| *Helicobacter pylori* infection | | | | | | | |
| Absent | 10 (50.0) | 25 (58.1) | 27 (56.3) | | 27 (48.2) | 35 (63.6) | |
| Present | 10 (50.0) | 18 (41.2) | 21 (43.8) | 0.83 | 29 (51.8) | 20 (36.4) | 0.07 |
| pT | | | | | | | |
| 1-2 | 7 (35.0) | 14 (32.6) | 13 (27.1) | | 21 (37.5) | 13 (23.6) | |
| 3-4 | 13 (65.0) | 29 (67.4) | 35 (72.9) | 0.76 | 35 (62.5) | 42 (76.4) | 0.15 |
| pN | | | | | | | |
| Negative | 5 (25.0) | 17 (39.5) | 14 (29.2) | | 23 (41.1) | 13 (23.6) | |
| Positive | 15 (75.0) | 26 (60.5) | 34 (70.8) | 0.42 | 33 (58.9) | 42 (76.4) | 0.07 |
| pM | | | | | | | |
| Negative | 18 (90.0) | 39 (90.7) | 37 (77.1) | | 51 (91.1) | 43 (78.2) | |
| Positive | 2 (10.0) | 4 (9.3) | 11 (22.9) | 0.15 | 5 (8.9) | 12 (21.8) | 0.07 |
| Stage | | | | | | | |
| I | 4 (20.0) | 12 (27.9) | 11 (22.9) | | 18 (32.2) | 9 (16.4) | |
| II | 4 (20.0) | 7 (16.3) | 4 (8.4) | | 8 (14.3) | 7 (12.7) | |
| III | 10 (50.0) | 20 (46.5) | 22 (45.8) | | 25 (44.6) | 27 (49.1) | |
| IV | 2 (10.0) | 4 (9.3) | 11 (22.9) | 0.48 | 5 (8.9) | 12 (21.8) | 0.11 |
| Serosal invasion | | | | | | | |
| Absent | 7 (35.0) | 14 (32.6) | 13 (27.1) | | 21 (37.5) | 13 (23.6) | |
| Present | 13 (65.0) | 29 (67.4) | 35 (72.9) | 0.76 | 35 (62.5) | 42 (76.4) | 0.15 |
| Vascular invasion | | | | | | | |
| Absent | 11 (55.0) | 18 (41.9) | 18 (37.5) | | 30 (53.6) | 17 (30.9) | |
| Present | 9 (45.0) | 25 (58.1) | 30 (62.5) | 0.41 | 26 (46.4) | 38 (69.1) | **0.02** |
| DNA ploidy | | | | | | | |
| Diploid | - | - | - | | 12 (21.4) | 8 (14.6) | |
| Monoclonal | | | | | 27 (48.2) | 16 (29.1) | |
| Multiclonal | | | | | 17 (30.4) | 31 (56.3) | **0.02** |
| Vital status at 7 years | | | | | | | |
| Alive | 9 (45.0) | 18 (41.9) | 17 (35.4) | | 25 (44.6) | 19 (34.6) | |
| Dead | 11 (55.0) | 25 (58.1) | 31 (64.6) | 0.71 | 31 (55.4) | 36 (65.4) | 0.28 |
| Total | 20 | 43 | 48 | | 56 | 55 | |

D: Diploid; A-mon: aneuploid monoclonal; A-mul: aneuploid multiclonal; SPF: S-phase fraction; low ≤9.6; high >9.6. †Fisher's exact test or Chi-square for trend, as appropriate.

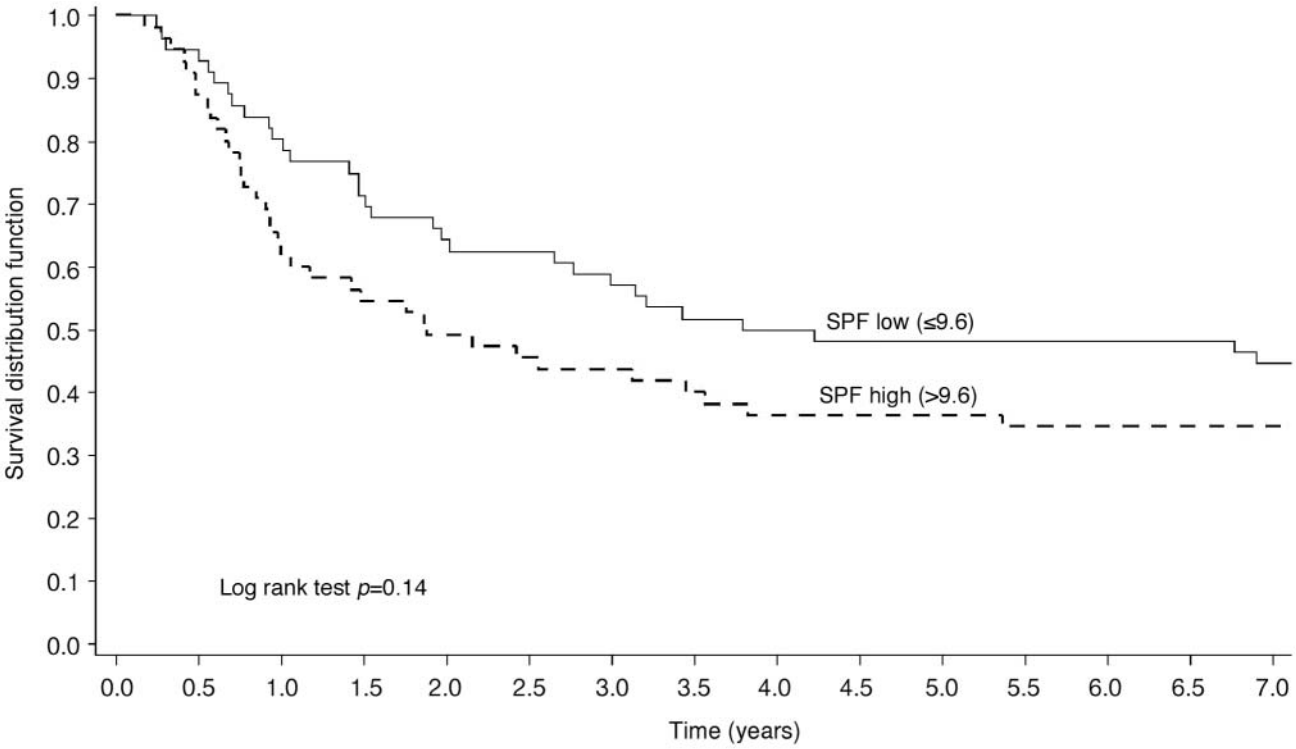


Figure 1. *Kaplan Meier 7-year survival curves of 111 GC cases according to SPF.*

## Discussion

Data from the literature suggest that the measurement of DNA nuclear content and SPF by means of FC may help identify patients with gastric carcinoma who have different risks of death or disease relapse (16-21). Nevertheless, these studies are often hampered by the heterogeneity in the composition and size of the series examined, as well as by the lack of uniformity in the methods of investigation (*i.e.* extraction of DNA from fresh or paraffin-embedded tissues). Most clinical studies have been conducted on archive material fixed in formalin and embedded in paraffin, regardless of the modality of sampling. The reliability of the results obtained is, therefore, debatable. In particular, loss of entire aneuploid cell lines following paraffin embedding has been reported. Moreover, tumour DNA nuclear content is hardly considered representative on the grounds of a single sample (22-24).

In our experience, investigations carried out on fresh tissue and with multiple sampling of single lesions permit the correct acquisition of cytometric data and the accurate assessment of the incidence of aneuploidy. Furthermore, multiple sampling provides evidence of several clones with different DNA content in the specimens taken from the same tumour and allows the prognostic significance of DI to be evaluated, along with that of tumour heterogeneity.

A great number of cells can be automatically processed by FC, which is also a reliable method for evaluating cell cycle kinetics more rapidly and cheaply than immunohistochemistry (Ki-67, PCNA) or *in vivo* bromodeoxyuridine labelling (6, 8).

In our series, the detection rate of aneuploidy (82.0%) is one of the highest reported in the literature so far. This is probably due to the sampling method used, multiple whenever possible, and to the preservation technique used for surgical specimens. Indeed, the analysis of fresh tissue compared to paraffin-embedded samples greatly reduces the probability of missing aneuploid clones, at the same time obtaining better histogram resolution.

We found no association between FC variables and clinicopathological features, *i.e.* tumour site and histotype, these findings being consistent with reports from other studies (25). A close association between multiclonal tumours and high SPF was demonstrated, suggesting that heterogeneity has a role in tackling host immunoreactivity. Indeed, the disturbance of the cell cycle due to increased proliferative activity of aneuploid clones or to a prolonged S-phase may increase the risk of genetic alteration (22, 26). This circumstance may generate DNA-aneuploid cell populations, which would explain the relationship between the high SPF and aneuploidy observed in the present study.

Table III. *Survival analysis at 7 years of 111 GC cases.*

| Clinicopathological variable | *P*-value | HR | 95% CI |
|---|---|---|---|
| Location (antrum) | **0.0004** | 0.41 | 0.25-0.67 |
| Macroscopic type (advanced *vs.* early) | **0.001** | 5.49 | 1.98-15.22 |
| Histological grade (poorly *vs.* moderately/well) | **0.0008** | 2.55 | 1.47-4.40 |
| Laurén classification (diffuse *vs.* other types) | **0.009** | 1.98 | 1.18-3.30 |
| Ming classification (infiltrative *vs.* expanding) | **0.006** | 2.88 | 1.36-6.09 |
| *Helicobacter pylori* infection | **0.03** | 0.57 | 0.34-0.95 |
| pT (3-4 *vs.* 1-2) | **0.0001** | 8.92 | 3.83-20.81 |
| pN (pos. *vs.* neg.) | **0.0001** | 6.83 | 3.33-14.01 |
| pM (pos. *vs.* neg.) | **0.0001** | 4.45 | 2.46-8.07 |
| Serosal invasion (pos. *vs.* neg.) | **0.0001** | 8.93 | 3.83-20.81 |
| Vascular invasion (pos. *vs.* neg.) | **0.0001** | 3.62 | 2.07-6.32 |
| DNA ploidy (diploid *vs.* monoclonal *vs.* multiclonal) | 0.75 | 1.05 | 0.75-1.49 |
| SPF (high *vs.* low) | **0.025** | 1.79 | 1.08-2.97 |

HR: Hazard ratio; 95% CI: 95% confidence interval using a Cox regression model including terms for all parameters listed in the table.

Table IV. *Multivariate survival analysis at 7 years of 111 GC cases.*

| Clinicopathological variable | *P*-value | HR | 95% CI |
|---|---|---|---|
| Gender (female) | 0.84 | 1.06 | 0.60-1.85 |
| Age at diagnosis (±median) | **0.006** | 2.14 | 1.24-3.69 |
| Location (antrum) | **0.04** | 0.56 | 0.32-0.98 |
| Ming classification (infiltrative type) | 0.08 | 2.00 | 0.92-4.36 |
| pT (3-4 *vs.* 1-2) | **0.01** | 6.58 | 1.52-28.44 |
| pN (pos. *vs.* neg.) | **0.028** | 2.68 | 1.11-6.46 |
| SPF (high *vs.* low) | 0.11 | 1.57 | 0.90-2.76 |
| DNA ploidy (diploid *vs.* monoclonal *vs.* multiclonal) | 0.28 | 0.81 | 0.54-1.20 |

HR: Hazard ratio; 95% CI: 95% confidence interval using a Cox regression model including terms for all parameters listed in the table.

In our investigation, overall survival is comparable with other Western series of resected gastric carcinomas (27), and the median follow-up of patients showing no relapse is longer than the median survival of patients eventually dying from the disease. DNA ploidy did not provide prognostic information, while SPF proved to be a more useful indicator of aggressive behaviour in patients with GC.

Data concerning the prognostic significance of SPF in GC are controversial (5, 6, 21, 25). Although several studies found univariate prognostic significance for survival even at different cut-offs, others could not confirm these findings. Russo *et al.* demonstrated multivariate independent significance for the SPF and chose a cut-off value at 15.2% (19). Lee *et al.* reported that tumours with a high SPF showed a shorter median survival than those with a low SPF (3), though Danesi *et al.* showed that SPF values had no effect on prognosis (28).

In our series, there was a trend for poorer survival of patients with highly proliferating tumours and the overall survival rate decreased from 44.6% in patients having tumours with low SPF to 34.6% for those with higher SPF, although this was not statistically significant. In a multivariate regression analysis adjusted also for sex and age, SPF emerged as a significant prognostic factor, with an increased risk of death for those patients with tumours with high SPF values. However, when the well known predictors of survival, such as pT and pN, were included in the model, the effect of SPF disappeared. It is questionable, however, whether prognostic independent significance would result from the observation of a greater number of patients over a longer period.

In conclusion, our results indicate that, rather than ploidy status, SPF might be a more useful predictor of survival in patients with GC. Further studies are necessary to confirm the prognostic value of SPF in gastric carcinoma and agreement needs to be reached on the criteria for SPF calculation, especially when SPF values are obtained under different conditions.

## References


1 Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. CA Cancer J Clin *55*: 74-108, 2005.

2 AIRT Working Group: Italian cancer figures – Report 2006. Incidence, mortality and estimates. Epidemiologia & Prevenzione *30(1), Suppl 2*: 34, 2006.

3 Lee KH, Lee JS, Lee JH, Kim SW, Suh C, Kim WK, Kim SH, Min YI, Kim BS, Park KC, Lee MS and Sun HS: Prognostic value of DNA flow cytometry in stomach cancer: a 5-year prospective study. Br J Cancer *79*: 1727-1735, 1999.

4 Nanus DM, Kelsen DP, Niedzwiecki D, Chapman D, Brennan M, Cheng E and Melamed M: Flow cytometry as a predictive indicator in patients with operable gastric cancer. J Clin Oncol *7*: 1105-1112, 1989.

5 Yonemura Y, Ooyama S, Sugiyama K, Kamata T, De Aretxabala X, Kimura H, Kosaka T, Yamaguchi A, Miwa K and Miyazaki I: Retrospective analysis of the prognostic significance of DNA ploidy patterns and S-phase fraction in gastric carcinoma. Cancer Res *50*: 509-514, 1990.

6 Victorzon M, Roberts PJ, Haglund C, von Boguslawsky K and Nordling S: Ki-67, ploidy and S-phase fraction as prognostic factors in gastric cancer. Anticancer Res *17*: 2923-2926, 1997.

7 Filipe MI, Rosa J, Sandey A, Imrie PR, Ormerod MG and Morris RW: Is DNA ploidy and proliferative activity of prognostic value in advanced gastric carcinoma? Hum Pathol *22*: 373-378, 1991.

8 Sendler A, Gilbertz KP, Becker I, Mueller J, Berger U, Fink U, van Beuningen D and Siewert JR: Proliferation kinetics and prognosis in gastric cancer after resection. Eur J Cancer *37*: 1635-1641, 2001.

9 Lauren PA and Nevalainen TJ: Epidemiology of intestinal and diffuse types of gastric carcinoma. A time-trend study in Finland with comparison between studies from high- and low-risk areas. Cancer *71*: 2926-2933, 1993.

10 Ming SC: Gastric carcinoma: a pathobiological classification. Cancer *39*: 2475-2485, 1977.

11 Japanese Research Society for Gastric Cancer: The general rules for the gastric cancer study in surgery and pathology. Jpn J Surg *11*: 127-145, 1981.

12 WHO classification of tumours – pathology and genetics of tumours of the digestive system. Hamilton SR and Aaltonen LA, editors. Lyon, IARC Press: 38-52, 2000.

13 American Joint Committee on Cancer: AJCC Cancer Staging Manual, 6th edition. New York, Springer-Verlag Publisher, pp. 99-106, 2002.

14 Dean PN and Jett JH: Mathematical analysis of DNA distribution derived from flow cytometry. J Cell Biol *60*: 523-527, 1974.

15 Rabinovitch PS: Numerical compensation for the effects of cell clumping on DNA histograms. Cytometry *4*: 27, 1990.

16 Danova M, Riccardi A, Mazzini G, Wilson G, Dionigi P, Brugnatelli S, Fiocca R, Ucci G, Jemos V and Ascari E: Flow cytometric analysis of paraffin-embedded material in human gastric cancer. Anal Quant Cytol Histol *10*: 200-206, 1988.

17 Johnson H Jr, Belluco C, Masood S, Abou-Azama AM, Kahn L and Wise L: The value of flow cytometric analysis in patients with gastric cancer. Arch Surg *128*: 314-317, 1993.

18 Sakusabe M, Kodama M, Sato Y, Kikuchi T and Koyama K: Clinical significance of DNA ploidy pattern in stage III gastric cancer. World J Surg *20*: 27-31, 1996.

19 Russo A, Bazan V, Migliavacca M, Zanna I, Tubiolo C, Tumminello FM, Dardanoni G, Cajozzo M, Bazan P, Modica G, Latteri M, Tomasino RM, Colucci G, Gebbia N and Leto G: Prognostic significance of DNA ploidy, S-phase fraction, and tissue levels of aspartic, cysteine, and serine proteases in operable gastric carcinoma. Clin Cancer Res *6*: 178-184, 2000.

20 Cascinu S, Graziano F, Catalano V, Valentini M, Rossi MC, Baldelli AM, Ghiselli R, Saba V, Giordani P and Catalano G: Prognostic value of S-phase fraction in T3N0M0 gastric cancer: implications for adjuvant therapy. Anticancer Res *20*: 3839-3842, 2000.

21 Esteban F, de Vega DS, Garcia R, Rodriguez R, Manzanares J, Almeida A and Tamames S: DNA content by flow cytometry in gastric carcinoma: pathology, ploidy and prognosis. Hepatogastroenterology *46*: 2039-2043, 1999.

22 Baba H, Korenaga D, Kakeji Y, Haraguchi M, Okamura T and Maehara Y: DNA ploidy and its clinical implications in gastric cancer. Surgery *131*: S63-70, 2002.

23 Dressler LG and Bartow SA: DNA flow cytometry in solid tumors: practical aspects and clinical applications. Semin Diagn Pathol *6*: 55-82, 1989.

24 Osterheld MC, Caron L, Demierre M, Laurini R and Bosman FT: DNA-ploidy in advanced gastric carcinoma is less heterogeneous than in early gastric cancer. Cell Oncol *26*: 21-29, 2004.

25 Jiao YF, Sugai T, Suzuki M, Uesugi N, Habano W, Nakamura S, Takagane A, Suto T and Yoshida T: Application of the crypt isolation technique to the flow cytometric analysis of DNA content in gastric carcinoma. Hum Pathol *35*: 587-593, 2004.

26 Sugai T, Habano W, Jiao YF, Suzuki M, Takagane A and Nakamura S: Analysis of genetic alterations associated with DNA diploidy, aneuploidy and multiploidy in gastric cancers. Oncology *68*: 548-557, 2005.

27 Lello E, Furnes B and Edna TH: Short and long-term survival from gastric cancer. A population-based study from a county hospital during 25 years. Acta Oncol *46*: 308-315, 2007.

28 Danesi DT, Spano M, Fabiano A, Altavista P, Pasqualetti P, Toscano MG, Antonini F, Catalano P, Mecozzi A, Picconi A, Daffina A and Cucchiara G: Flow cytometric DNA ploidy, p53, PCNA, and c-erbB-2 protein expressions as predictors of survival in surgically resected gastric cancer patients. Cytometry *42*: 27-34, 2000.


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