9. Zedeler K. Assessment and presentation of survival experience in the Danish Breast Cancer Cooperative Group. *Acta Oncol* 1988, **27**, 649–662.
10. Cox DR. Regression models and life-tables. *J Royal Stat Soc* 1972, 187–200.
11. Andersen PK, Væth M. *Statistisk Analyse af Overlevelsesdata ved Lægevidenskabelige Undersøgelser*. Copenhagen, FADL's Forlag, 1984.
12. Wargotz ES, Silverberg SG. Medullary carcinoma of the breast: a clinicopathologic study with appraisal of current diagnostic criteria. *Human Pathol* 1988, **19**, 1340–1346.
13. Rapin V, Contesso G, Mouriesse H, *et al*. Medullary breast carcinoma. *Cancer* 1988, **61**, 2503–2510.
14. Kurtz JM, Jacquemier J, Torhorst J, *et al*. Conservation therapy for breast cancers other than infiltrating ductal carcinoma. *Cancer* 1989, **63**, 1630–1635.
15. Fisher ER, Redmond C, Fisher B, Bass G. Pathologic findings from the National Surgical Adjuvant Breast and Bowel Projects (NSABP). *Cancer* 1990, **65**, 2121–2128.
16. Foote FW, Stewart FW. A histologic classification of carcinoma of the breast. *Surgery* 1946, **19**, 74–99.
17. Moore OS Jr, Foote FW. The relatively favorable prognosis of medullary carcinoma of the breast. *Cancer* 1949, **2**, 635–642.
18. Mitze M, Goepel E. Prognostische Faktoren beim medullären Mammakarzinom. *Geburtsh u Frauenheilk* 1989, **49**, 635–641.
19. Carter D, Elkins RC, Pipkin RD, Abbey H, Shepard RH. Relationship of necrosis and tumor border to lymph node metastases and 10-year survival in carcinoma of the breast. *Am J Surg Pathol* 1978, **2**, 39–46.
20. Schiødt T. *Breast Carcinoma*. Copenhagen, Munksgaard, 1966.
21. Gorski CM, Niepolomska W, Nowak K, *et al*. Clinical evaluation and pathological grading in relation to other prognostic factors. In Forrest APM, Kunkler PB, eds. *Prognostic Factors in Breast Cancer*. Baltimore, Williams and Wilkins, 1968, 309–318.
22. Schwartz GF. Solid circumscribed carcinoma of the breast. *Ann Surg* 1969, **169**, 165–173.
23. Haagensen CD. *Diseases of the Breast*. Philadelphia, W.B. Saunders (2nd edn), 1973.

Pergamon

European Journal of Cancer Vol. 30A, No. 12, pp. 1797–1803, 1994

0959–8049/94 $7.00+0.00

0959-8049(94)E0159-2

Histological Grade, Perineural Infiltration, Tumour-infiltrating Lymphocytes and Apoptosis as Determinants of Long-term Prognosis in Prostatic Adenocarcinoma

S. Vesalainen, P. Lipponen, M. Talja and K. Syrjänen

A series of 325 prostatic adenocarcinomas with a long-term clinical follow-up were subjected to light microscopic analysis of histological prognostic factors, including three grading systems (Gleason score, WHO grade, nuclear grade), perineural infiltration of the tumour (PNI), tumour infiltrating lymphocytes (TIL) and the presence of apoptotic cells (APO). All three histological classifications correlated significantly with the prognosis, but in multivariate analysis, the Gleason score was superior to the WHO grading or nuclear grading in predicting patient survival. PNI was significantly related to poor differentiation of the tumour, its progression and ominous disease outcome, particularly in T1–2MO tumours. The density of TIL was independent of the tumour differentiation, and absent or weak TIL were signs of a high risk of tumour progression and of a fatal disease. Apoptotic cells were commonly detected in poorly differentiated tumours and apoptosis was related to disease progression and low survival probability. The results suggest that the Gleason score, PNI and the density of TIL should be included in routine pathology reports, to be used by clinicians while making therapeutic decisions in prostatic cancer.

Key words: prostatic adenocarcinoma, Gleason score, WHO grade, nuclear grade, perineural infiltration, apoptosis, tumour infiltrating lymphocyte, progression, survival
Eur J Cancer, Vol. 30A, No. 12, pp. 1797–1803, 1994

INTRODUCTION

PROSTATIC ADENOCARCINOMA is a clinically and biologically heterogeneous disease [1–6]. A proportion of tumours run an unfavourable course, while the majority of the tumours do not lead to death [6]. A number of histological grading systems have been introduced in prostatic adenocarcinoma to predict the disease outcome and to assist in selecting the proper therapy [1, 7, 8]. However, opinions differ as to which of the grading systems gives the best prognostic estimates. The Gleason score is based on histological architectural features alone [1], while the WHO grading system divides tumours into three malignancy grades on the basis of architectural and cellular features [7].

Prognostic evaluation of the tumours can also be based on cellular features alone. Nuclear grading omits the histological architecture of the tumours and is based on nuclear characteristics alone [9, 10].

Prognosis of the prostatic tumours is related to specific histological features, in addition to histological differentiation. The potential to invade into the surrounding tissues [11, 12] or inflammatory cell reaction (tumour infiltrating lymphocytes, TIL) [13, 14] in the tumour stroma are known to be of prognostic significance. Perineural infiltration (PNI) of the tumour is typical for prostatic cancer and it also bears some prognostic information [12, 15]. The density of TIL has been related to prognosis in several epithelial tumours [13, 14], but in prostatic carcinoma, no previous reports are available on the eventual prognostic value of this histological feature.

Programmed cell death (apoptosis) is characterised by nuclear fragmentation [16–20], which can be easily identified in prostatic lesions as well. The prognostic significance of cell proliferation is already established in prostatic tumours [2, 4], whereas the prognostic significance of apoptosis is unknown.

The present study was designed to assess the prognostic value of three subjective grading systems and three special histological features in a cohort of 325 patients with prostatic adenocarcinoma followed-up for over 13 years. The prognostic potential of these histological features was compared to the clinical prognostic factors in a multivariate analysis.

PATIENTS AND METHODS

325 patients with prostatic adenocarcinoma were diagnosed, treated and followed-up during 1971–1992 at the Department of Surgery, Kuopio University Hospital, Finland. The mean (S.E.) age of the patients at diagnosis was 71.5 (0.4) years (range 39.9–92.0). The follow-up period lasted for a mean (S.E.) of 13.0 (0.2) years (range 7.5–21.4). Diagnosis, clinical staging [21], treatment and follow-up were carried out mainly by two urologists according to standard clinical practice. During the first 5 years of the follow-up, the examinations were performed every 3–6 months and thereafter approximately once a year. This scheme was modified if necessary because of the activity of the disease. All tumours were treated according to the generally accepted principles. There were eight radical prostatectomies and four radical radiation therapies and 107 patients were treated by oestrogens. A combination of oestrogen and cytostatics was used in 4 cases. Orchiectomy was performed in 159 patients and transurethral resection in 85 patients. A total of 106 cases were subjected to active follow-up only without any primary therapy. There were 114 T1, 77 T2, 101 T3 and 33 T4 tumours. Of the 325 patients included in this analysis, 224 had no detectable metastases at the time of diagnosis, while 101 had a metastatic disease. The progression of tumours was defined as an increase in the T or M category during the follow-up. The N category was not used because it can be accurately defined only by pelvic lymph node dissection which was infrequently performed in our clinic during the study period. The causes of death were verified from patient files, death certificates and from the files of the Finnish Cancer Registry.

Correspondence to P. Lipponen.
S. Vesaleinen and M. Talja are at the Department of Surgery; and P. Lipponen and K. Syrjänen are at the Department of Pathology and Forensic Medicine, University of Kuopio, POB 1627, FIN-70211 Kuopio, Finland.
Received 18 Jan. 1994; accepted 7 Apr. 1994.

Histological methods

Five-micrometre thick sections were cut from the paraffin-embedded surgical and needle tumour specimens and stained with hematoxylin and eosin. The samples were examined for the histological prognostic factors by one investigator who was unaware of the clinical data. Tumours were graded according to the grading system of Gleason [1] and that of the WHO [7]. Nuclear grading (NG) of the tumours was completed into four nuclear grades, as detailed in the previous literature [9, 10]. Perineural infiltration (PNI) was categorised into two groups: no (0) and yes (1).

The presence of apoptotic cells in the sections was also scored into two groups; no (0) and yes (1). The criteria used in defining apoptosis were adopted from the literature [16]. These include the following: (a) cells showing marked condensation of chromatin and cytoplasm, (b) cytoplasmic fragments containing condensed chromatin, (c) intra- and extracellular chromatin fragments down to diameter of about 2 μm.

The density of TILs was scored into three groups as detailed previously [13, 14]. In TIL grade 1, lymphocytes were rare or absent. A TIL 2 category included tumours with a moderate lymphocyte infiltrate, and TIL grade 3, tumours with a dense lymphocyte infiltrate.

Statistical methods

In basic statistical calculations, the SPSS-X was used in an IBM computer and the statistical tests used are indicated in results when appropriate. Frequency distributions were tested by the χ^2 test and Yate's correction was applied when appropriate. The univariate survival analysis (log-rank analysis) was based on the life-table method with the statistics by Lee and Desu [22]. Multivariate survival analysis was carried out with the BMDP (2L) [23] in a stepwise manner. Both survival analyses used deaths from prostatic carcinoma as events. Progression-free survival was defined as the time interval between the primary therapy and the first signs of progression of the disease (T or M category). In multivariate analysis, the enter limit was $P < 0.1$ and the removal limit was $P > 0.15$.

RESULTS

The distribution of cases into different malignancy grades is shown in Table 1. The results show that the nuclear grade, WHO grade and Gleason score are significantly interrelated. The density of TIL was independent of the Gleason score while apoptosis and perineural infiltration were significantly more common in Gleason score 8–10 tumours than in those with Gleason score values between 2 and 4.

Progression of T1–2MO tumours was significantly related to the Gleason score, WHO grade and nuclear grade (Table 2). The presence of apoptotic cells predicted progression in T and M categories, whereas the PNI and the density of TIL were related significantly to progression in the M category only (Table 2).

The survival analysis showed that all the analysed histological features were significantly related to prognosis in T1–2MO (Table 3, Figures 1–4), MO and in T1–4MO-1 tumours (Table 3, Figures 5, 6). The same features also predicted the progression-free survival (Table 4). According to the univariate survival analysis, Gleason score, WHO grade, PNI and the presence of apoptic cells were the most important prognostic factors, in addition to TM classification.

In the multivariate analysis, M category, Gleason score, T category and patient age were independent prognostic factors in that order (Table 5). In MO tumours, T category, patient

Table 1. Gleason score related to other histological features in prostatic adenocarcinoma

Variable	n	Gleason score 2–4	5–7	8–10	χ^2	P*
WHO grade 1	11	6	5	0		
WHO grade 2	117	11	100	6	207	< 0.0001
WHO grade 3	197	1	44	152		
NG 1	11	6	5	0		
NG 2	116	11	99	6	224	< 0.0001
NG 3	127	1	43	83		
NG 4	71	0	2	69		
TIL 1	131	6	52	74		
TIL 2	127	7	63	57	5	0.2
TIL 3	66	5	34	27		
APO 0	167	18	124	25	157	< 0.0001
APO 1	158	0	25	133		
PNI 0	107	7	76	24	56	< 0.0001
PNI 1	77	0	17	60		

*χ^4 test. PNI, perineural infiltration; TIL, tumour infiltrating lymphocytes; APO, apoptosis; NG, nuclear grading.

Table 2. Progression of T1–2MO tumours as related to histological features

Variable	Progression in T category No	Yes	χ^2 P*	Progression in M category No	Yes	χ^2 P*
Gleason 2–4	13	0	10	12	1	16
Gleason 5–7	91	19	0.006	92	13	< 0.001
Gleason 8–10	27	15		27	18	
WHO grade 1	5	2	5	8	0	117
WHO grade 2	74	12	0.087	74	7	0.0001
WHO grade 3	52	20		49	25	
NG 1	5	2		8	0	
NG 2	74	11	8	73	7	30
NG 3	46	16	0.036	46	16	< 0.001
NG 4	6	5		4	9	
PNI 0	61	15	1	66	9	7
PNI 1	18	8	0.2	17	9	0.009
TIL 1	36	11	1	30	15	8
TIL 2	61	14	0.8	66	9	0.016
TIL 3	34	9		35	8	
APO 0	98	19	5	101	14	14
APO 1	33	15	0.030	14	18	0.002

*χ^2 test. See Table 1 for abbreviations.

age, Gleason score and the density of TIL had independent prognostic significance (Table 5). In T1-2MO tumours, histological differentiation, PNI, the density of TIL and T category were independent prognostic factors (Table 5).

DISCUSSION

Clinical stage is the most important prognostic factor in prostatic adenocarcinoma, and in particular, the presence of distant metastasis at diagnosis refers to a high intrinsic malignancy [2, 4]. The survival rate of T1–2MO tumours was high which indicates that their malignant potential and the ability to send metastases are low. Local tumours are usually diploid and slowly proliferating [2–5] and alterations in oncogenes are rare [4]. In contrast, in invasive prostatic tumours, aneuploidy is a common feature [2], they are rapidly proliferating [2] and changes in oncogenes are often present [4]. In general, survival rates of the patients in the various stage categories of the present series are consistent with the results presented in the previous literature [1–4, 24, 25].

Histological differentiation is a key determinant of prognosis and therapy in prostatic tumours [1, 7, 8, 12]. This analysis showed that the Gleason score [1] was superior to the other classification systems in predicting prognosis [7–10]. The Gleason score is based on the assessment of different aspects of malignant architectural features which generally improves the prognostic value of a given prognostic parameter, e.g. grade as it also does in breast cancer [26]. However, the Gleason score has not been widely used in the clinical context due to its

Table 3. Ten-year survival of patients with T1–2MO, and T1–4MO–1 tumours categorised according to clinical and histological prognostic factors

Variable	T1–2MO n	T1–2MO Survival (%) at 10 years	T1–2MO χ^2 P*	T1–4MO–1 n	T1–4MO–1 Survival (%) at 10 years	T1–4MO–1 χ^2 P*
T1	110	85	18.5	113	85	91.8
T2	63	60	< 0.0001	77	50	< 0.0001
T3		—		101	15	
T4		—		33	0	
MO		—		224	65	107.6
M1		—		101	5	< 0.0001
Gleason 2–4	14	100	13.3	18	95	50.1
Gleason 5–7	114	85	0.0013	149	70	< 0.0001
Gleason 8–10	46	50		158	25	
WHO 1	8	100	9.3	11	80	30.2
WHO 2	89	85	0.0095	117	70	< 0.0001
WHO 3	77	60		197	30	
NG 1	8	100	13.8	11	80	37.1
NG 2	88	85	0.0050	116	70	< 0.0001
NG 3	65	70		127	40	
NG 4	13	40		71	15	
PNI 0	82	90	15.5	107	65	19.7
PNI 1	27	45	0.0001	77	25	< 0.0001
TIL 1	48	65	7.6	132	30	11.4
TIL 2	79	75	0.0218	127	55	0.0033
TIL 3	47	90		66	70	
APO 0	133	85	10.4		65	28.3
APO 0	51	55	0.0012		30	< 0.0001

*Log-rank analysis. See Table 1 for abbreviations.

relatively complex assessment as compared to the WHO grade or nuclear grade [7, 9, 10, 15]. The present results clearly show that the Gleason score should be adopted in routine use in the pathology laboratories, since it is more closely related to patient survival than the WHO grade or nuclear grade.

Nuclear grading is based on the identification of various nuclear features such as the size, shape, chromatin structure and the presence of nucleoli in cancer cells [9, 10]. The nuclear grade closely correlates to prognosis in several human tumours [9, 10, 27], and the present results are in alignment with the previous reports. The results suggest that nuclear grading can

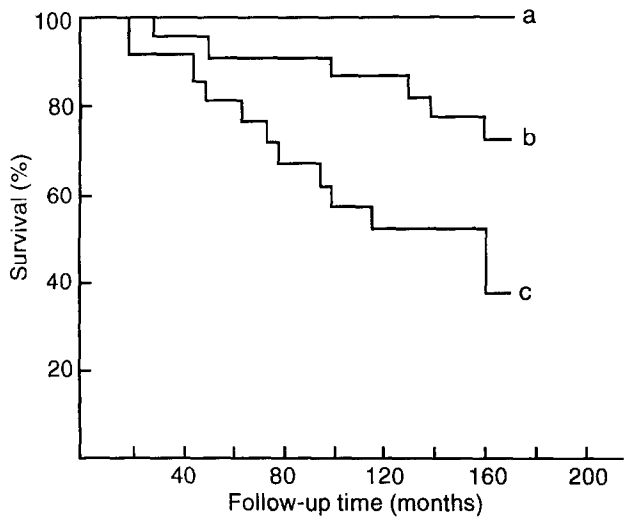

Figure 1. Survival of the patients with a T1–2MO tumour subdivided according to the Gleason score. The difference in survival among the curves is significant (χ^2 = 13.2, P = 0.0013). Curve a: Gleason score 2–4, n = 14; curve b: Gleason score 5–7, n = 114; curve c: Gleason score 8–10, n = 46.

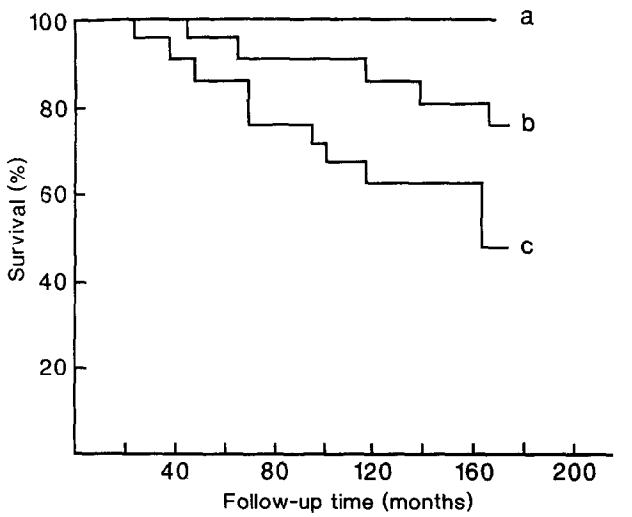

Figure 2. Survival of the patients with a T1–2MO tumour subdivided according to the WHO grade. The difference in survival among the curves is significant (χ^2 = 9.3, P = 0.0095). Curve a: grade 1, n = 8; curve b: grade 2, n = 89; curve c: grade 3, n = 77.

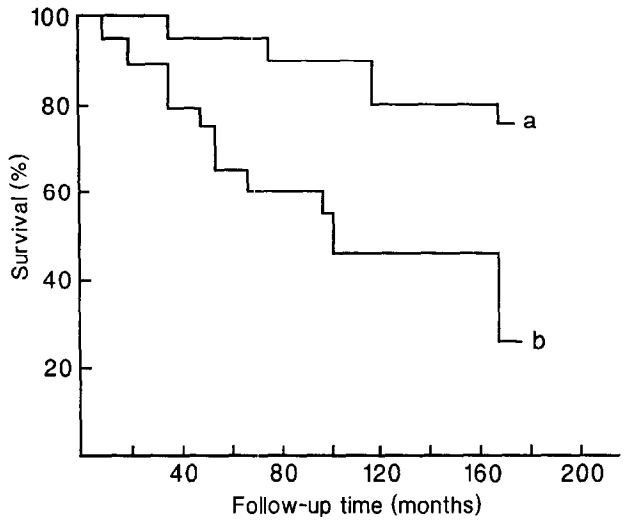

Figure 3. Survival of the patients with a T1–2MO tumour subdivided according to perineural infiltration (PNI). The difference in survival between the curves is significant ($\chi^2 = 15.5$, $P = 0.0001$). Curve a: PNI(−), $n = 82$; curve b: PNI(+), $n = 27$.

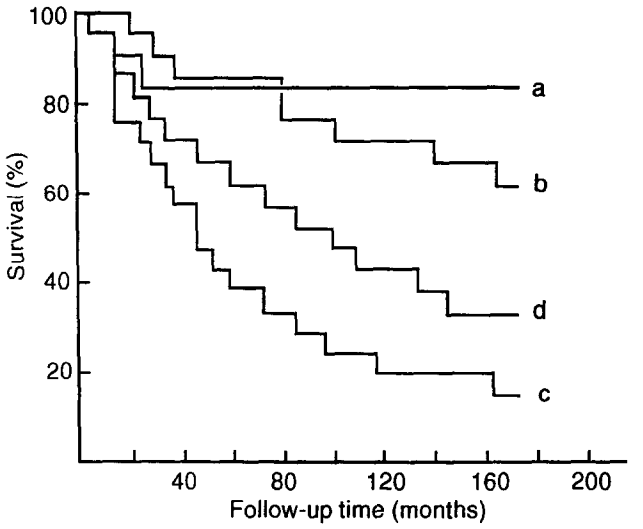

Figure 5. Survival of the patients with a T1–4MO–1 tumour subdivided according to the nuclear grade (NG). The difference in survival among the curves is significant ($\chi^2 = 31.7$, $P < 0.0001$). Curve a: NG1, $n = 11$; curve B: NG2, $n = 116$; curve c: NG3, $n = 127$; curve d: NG4, $n = 71$.

be used in prostatic adenocarcinoma, especially in cases where the amount of tissue available for grading is limited, e.g. in needle biopsy specimens. In particular, disease progression and poor outcome are highly probable in nuclear grade 3–4 tumours, while the difference in prognosis between the nuclear grade 1 and 2 tumours is not dramatic.

PNI has been previously related to malignancy in prostatic adenocarcinoma [12, 15] which was confirmed by the present results. PNI is usually related to malignant cellular features and rapid cell proliferation which are all general signs of a high degree of malignancy [12]. In particular, PNI seems to have prognostic significance in local T1-2MO tumours, and accordingly, it should be included in the routine histopathology reports. However, PNI cannot always be assessed due to a limited amount of tissue available for diagnostic purposes, i.e. in needle biopsies.

The density of TIL has been previously related to prognosis in various neoplasms [12–14]. The majority of the lymphocytes infiltrating human tumours are T-lymphocytes [28], including the cytotoxic cells which may resist tumour growth and promote a higher survival rate. In breast cancer, tumours with a dense TIL have a better prognosis than in those without any lymphocyte reaction [13]. Similarly, in urological neoplasia (bladder and renal carcinomas), dense TIL refers to a higher survival probability [12, 14]. The present analysis shows that also in prostatic adenocarcinoma, tumour–host interactions have prognostic significance since tumours with a dense TIL have a

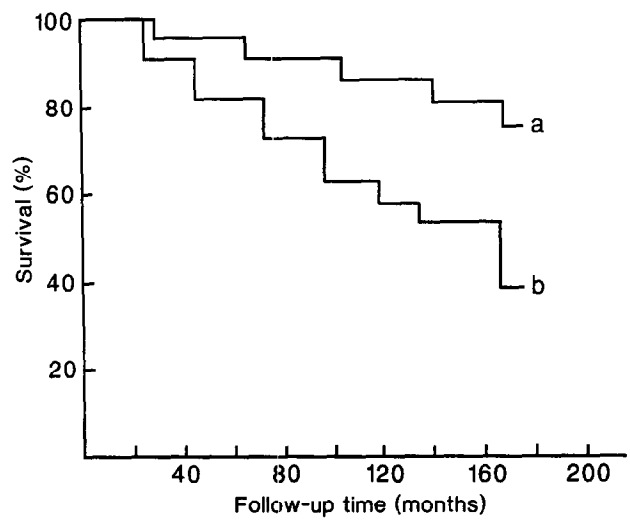

Figure 4. Survival of the patients with a T1–2MO tumour subdivided according to the presence of apoptotic nuclei (APO). The difference in survival between the curves is significant ($\chi^2 = 10.4$, $P = 0.0012$). Curve a: APO(−), $n = 133$; curve b: APO(+), $n = 51$.

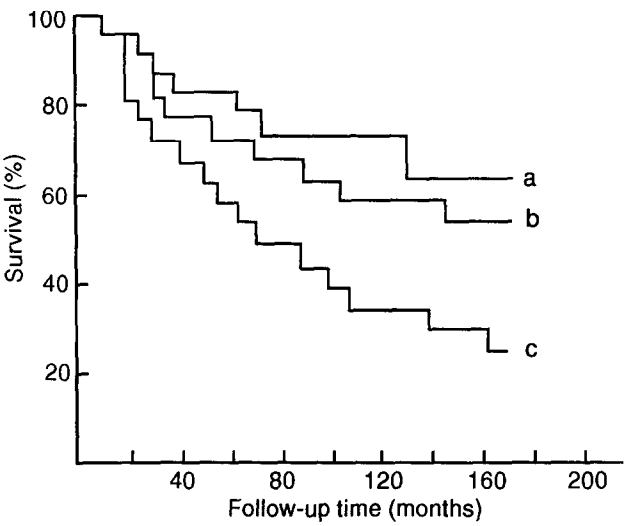

Figure 6. Survival of the patients with a T1–4MO–1 tumour subdivided according to the tumour infiltrating lymphocyte (TIL) grade. The difference in survival among the curves is significant ($\chi^2 = 11.4$, $P = 0.0033$). Curve a: TIL(3), $n = 66$; curve b: TIL(2), $n = 127$; curve c: TIL(1), $n = 132$.

Table 4. Ten-year progression-free survival (PFS) and survival (S) of patients with MO tumours categorised according to histological prognostic factors

Variable	*n*	PFS at 10 years (%)	χ^2 *P*	S at 10 years (%)	χ^2 *P*
Gleason 2–4	15	95	34.2	100	23.1
Gleason 5–7	126	85	< 0.0001	80	< 0.0001
Gleason 8–10	83	50		45	
WHO 1	8	90	19.9	100	18.8
WHO 2	97	85	< 0.0001	80	0.0001
WHO 3	119	60		55	
NG 1	8	90	24.5	100	24.4
NG 2	96	85	0.0001	80	0.0001
NG 3	90	65		60	
NG 4	30	40		30	
PNI 0	92	85	19.6	80	13.4
PNI 1	44	50	< 0.0001	45	0.0002
TIL 1	79	60	14.4	50	12.7
TIL 2	90	85	< 0.0007	75	0.0017
TIL 3	55	75		80	
APO 0	133	85	25.5	80	22.7
APO 1	91	55	< 0.0001	45	< 0.0001

See Table 1 for abbreviations.

30% higher survival probability in the univariate analysis than tumours with absent or weak TIL only.

Programmed cell death is a normal physiological process by which cells are eliminated during embryonic development and in adult life [17]. Apoptosis is characterised by a cascade of genetic and biochemical events that cause cell shrinkage, condensation of cytoplasmic and nuclear material, cleavage of chromosomal DNA into approximately 200 base pair fragments and enchanged recognition of the dying cells by phagocytes [16, 17, 28–30]. Disturbances in this physiological process may lead to the development of neoplastic diseases [18–20]. In an involuting prostate, apoptotic cells can be found, most probably related to changes in androgen metabolism during old age [31], since cells are dependent on various viability factors, like growth factors and hormones [31].

In cancer, apoptosis is under highly complex regulation and oncogenes (c-*myc*, *bcl*-2 and p53) are also involved in the process [17, 29, 30, 32] by regulating the direction of the cell cycle either towards mitosis or apoptosis. It is becoming clear that mitosis and apoptosis are linked in some way and that the balance achieved depends on signals received from appropriate growth or survival factors [19, 20]. The present results show that

Table 5. Independent predictors of survival in Cox's analysis at the significance level of $P < 0.05$

	β (S.E.)	*P*	Hazard rate (95% C.I.)
All cases			
M category	1.224 (0.222)	< 0.001	3.40 (2.18–5.30)
Gleason score	0.713 (0.191)	< 0.001	2.04 (1.39–2.98)
T category	0.421 (0.118)	0.001	1.52 (1.20–1.92)
Age	0.028 (0.011)	0.015	1.02 (1.01–1.05)
T1–4M0			
T category	0.802 (0.169)	< 0.001	2.23 (1.59–3.12)
Age	0.062 (0.016)	< 0.001	1.06 (1.03–1.09)
Gleason score	0.748 (0.285)	0.014	2.11 (1.19–3.74)
TIL grade	−0.391 (0.175)	0.012	0.67 (0.47–0.95)
T1–2MO*			
PNI	0.870 (0.475)	< 0.001	2.38 (0.92–6.17)
T category	0.841 (0.462)	0.026	2.31 (0.92–5.84)
TIL grade	−0.818 (0.322)	0.041	0.44 (0.23–0.84)

β, coefficient of the regression model. Hazard rate = e^{β}

*If perineural infiltration was not included, independent predictors were Gleason score (RR = 3.06, $P < 0.001$), T category (RR = 2.77, $P < 0.001$) and TIL grade (RR = 0.59, $P = 0.026$). C.I., confidence interval; TIL, tumour infiltrating lymphocytes; PNI, perineural infiltration; RR, relative risk.

apoptosis is related to a low differentiation grade, e.g. rapid cell proliferation. This relationship is reflected in the results of survival analysis which shows that the presence of apoptotic cells in the specimens refers to a poor disease outcome. These results are in line with the results obtained by direct measurement of cell proliferation in prostatic adenocarcinomas [2, 5]. However, more sophisticated methods are clearly needed to clarify the role of programmed cell death in prostatic neoplasia.

Multivariate analysis also included patient age to control its confounding effect since the prognostic significance of patient age has been recognised in several neoplasms [33, 34]. Also, in prostatic cancer, old men have a more unfavourable prognosis than do their younger counterparts and this applies to invasive tumours, in particular. The lower survival rate of old men is not due to TIL since TIL and age are not related significantly. It may be due to several comorbid diseases and compromised general condition, including a reduced capacity to resist tumour growth. In MO tumours, the host response (TIL) was a highly significant independent prognostic factor. The risk ratio between TIL grades ranged between 0.4 and 0.6 which means that the risk of death of prostatic adenocarcinoma in TIL grade 1 is over two times as high as in TIL grade 3 tumours. The risk ratios in prostatic adenocarcinoma are close to those found in the breast, bladder or renal tumours [12, 14] which suggests some generality in the role of tumour–host interactions in the prognosis of neoplastic diseases.

In summing up the results of this study, we conclude that Gleason score, PNI and the density of lymphocyte reaction in prostatic adenocarcinoma should be included in routine histopathology reports. These histological characteristics combined with patient age and extent of the primary tumour provide a bulk of prognostic information for the urologist to use in making therapeutic decisions.

1. Gleason DF. Histologic grading and clinical staging of prostatic carcinoma. In Tannenbaum, ed. *Urologic Pathology: The Prostate*. Philadelphia, Lea & Febiger, 1977, 171.
2. Visakorpi T, Kallioniemi O-P, Paronen IYI, Isola JJ, Heikkinen AI, Koivula TA. Flow cytometric analysis of DNA ploidy and S phase fraction from prostatic carcinomas: implications for prognosis and response to endocrine therapy. *Br J Cancer* 1991, **64**, 578–582.
3. Song J, Cheng WS, Cupps RE, Earle JD, Farrow GM, Lieber MM. Nuclear deoxyribonucleic acid content measured by static cytometry: important prognostic association for patients with clinically localized prostate carcinoma treated by external beam radiotherapy. *J Urol* 1992, **147**, 794–797.
4. Visakorpi T, Kallioniemi O-P, Heikkinen A, Koivula T, Isola J. Small subgroup of aggressive highly proliferative prostatic carcinomas defined by p53 accumulation. *J Natl Cancer Inst* 1992, **84**, 883–887.
5. Harper ME, Goddard G-JL, Wilson DW, *et al.* Relationship of proliferating cell nuclear antigen (PCNA) in prostatic carcinomas to various clinical parameters. *Prostate* 1992, **20**, 243–253.
6. Muir CS, Nectoux J, Staszewski J. The epidemiology of prostatic cancer. *Acta Oncol* 1991, **30**, 133–140.
7. Mostofi FK. Grading of prostatic carcinoma. *Cancer Chemother Rep* 1975, **59**, 111–117.
8. Gaeta JF, Asirwatham JE, Miller G, Murphy GP. Histologic grading of primary prostatic cancer; a new approach to an old problem. *J Urol* 1980, **123**, 689–693.
9. Black MM, Kerpe S, Speer FD. Survival in breast cancer cases in relation to the structure of the primary tumour and the regional lymph nodes. *Surg Gynecol Obstet* 1955, **100**, 543–550.
10. Syrjänen KJ, Hjelt LH. Ultrastructural characteristics of human renal-cell carcinoma in relation to light microscopic grading. *Scand J Urol Nephrol* 1978, **12**, 57–65.
11. Clayton F. Pathologic correlates of survival in 378 lymph node-negative infiltrating ductal breast carcinomas. *Cancer* 1991, **68**, 1309–1317.
12. Eskelinen MJ, Lipponen PK, Majapuro R, Syrjänen K. Prognostic factors in prostatic adenocarcinoma assessed by means of quantitative histology. *Eur Urol* 1991, **19**, 274–278.
13. Aaltomaa S, Lipponen PK, Eskelinen MJ, *et al.* Lymphocyte infiltrates as a prognostic variable in female breast cancer. *Eur J Cancer* 1992, **28A**, 859–864.
14. Lipponen PK, Eskelinen MJ, Jauhiainen K, Harju E, Terho R. Tumour infiltrating lymphocytes as an independent prognostic factor in transitional cell bladder cancer. *Eur J Cancer* 1993, **29A**, 69–75.
15. Olsen S. *Tumours of the Kidney and Urinary Tract*. Mungsgaard, 1983.
16. Walker NI, Harmon BV, Gobe GC, Kerr JFR. Patterns of cell death. *Meth Achiev Exp Pathol* 1988, **13**, 18–54.
17. Wyllie AH. Apoptosis and the regulation of cell numbers in normal and neoplastic tissues: an overview. *Cancer Metast Rev* 1992, **11**, 95–103.
18. Hollowood K, Macartney JC. Reduced apoptotic cell death in follicular lymphoma. *J Pathol* 1991, **163**, 337–342.
19. Allan DJ, Howell A, Roberts SA, *et al.* Reduction in apoptosis relative to mitosis in histologically normal epithelium accompanies fibrocystic change and carcinoma of the premenopausal human breast. *J Pathol* 1991, **163**, 337–342.
20. Huovinen R, Wärri A, Collan Y. Mitotic activity, apoptosis and TRPM-2 mRNA expression in DMBA-induced rat mammary carcinoma treated with anti-estrogen toremifene. *Int J Cancer* 1993, **55**, 685–691.
21. International Union against Cancer (UICC). *TNM Classification of Malignant Tumours*, 3rd edition. Geneva, UICC, 1978.
22. Lee E, Desu M. A computer program for comparing k samples with right censored data. *Comput Prog Biomed* 1972, **2**, 315–320.
23. Cox DR. Regression models and life tables with discussion. *J R Stat Soc B* 1972, **34**, 187–220.
24. Willson JWL, Morales A, Bruce AW. The prognostic significance of histological grading and pathological staging in carcinoma of the prostate. *J Urol* 1983, **130**, 481–483.
25. Forman JD, Order SE, Zinreich ES, Lee D-J, Wharam MD, Mellits ED. The correlation of pretreatment transurethral resection of prostatic cancer with tumor dissemination and disease-free survival. A univariate and multivariate analysis. *Cancer* 1986, **58**, 1770–1778.
26. Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer. *Br J Cancer* 1957, **11**, 359–377.
27. Eskelinen M, Lipponen P, Aitto-Oja L, Hall O, Syrjänen K. The value of histoquantitative measurements in prognostic assessment of renal adenocarcinoma. *Int J Cancer* 1993, **55**, 547–554.
28. Naukkarinen A, Syrjänen K. Quantitative immunohistochemical analysis of mononuclear infiltrates in breast carcinomas—correlation with tumour differentiation. *J Pathol* 1990, **160**, 217–222.
29. Eastman A. Apoptosis; a product of programmed and unprogrammed cell death. *Toxicol Appl Pharmacol* 1993, **121**, 160–164.
30. Sachs L, Lotem J. Control of programmed cell death in normal and leucemic cells: new implications for therapy. *Blood* 1993, **82**, 15–21.
31. Bowen ID. Apoptosis or programmed cell death? *Cell Biol Int* 1993, **17**, 365–380.
32. Colombel M, Olsson CA, Ng P-Y, Buttyan R. Hormone-regulated apoptosis results from re-entry of differentiated prostate cells onto defective cell cycle. *Cancer Res* 1992, **52**, 4313–4319.
33. Hockenbery DM. The *bcl-2* oncogene and apoptosis. *Semin Immunol* 1992, **4**, 413–420.
34. Aaltomaa S, Lipponen P, Eskelinen M. Demographic prognostic factors in breast cancer. *Acta Oncol* 1992, **31**, 635–640.
35. Bergman L, Dekker G, Van Leeuwen FE, Huisman SJ, Van Dam FSAM, Van Dongen JA. The effect of age on treatment choice and survival in elderly breast cancer patients. *Cancer* 1991, **67**, 2227–2234.

Acknowledgements—The study was supported by a research grant from the Savo Cancer Fund. The skilful technical assistance of Ms Irma Väänänen is gratefully acknowledged. The survival curves were kindly reproduced by Muotomania, Kuopio, Finland.