Introduction
Apoptosis and necrosis are the two basic types of cell death (Searle et al. 1982, Buja et al. 1993). Cell death occurring during embryonic development, during organogenesis, in several physiological processes during adult life and in pathophysiological conditions is known as apoptosis (Wyllie 1992, Buja et al. 1993). The pathogenesis of apoptosis involves the cleavage of nuclear chromatin between the nucleosomes by specific endonucleases, producing chromatin fragments composed of approximately 200 base pairs (Wyllie 1992, Buja et al. 1993). The apoptotic process is controlled by inducers and repressors, the balance between these stimuli determining whether the cell cycle enters mitosis or apoptosis (Wyllie 1992, Buja et al. 1993). According to recent hypotheses, clonal expansion might occur as a premalignant event resulting from suppressed apoptosis, secondary to mutations in the regulatory oncogenes. Of such oncogenes, at least Bcl-2 (Hockenbery 1992, van Slooten et al. 1998), c-myc (Koskinen & Alitalo 1993) and p53 (Wyllie 1992, van Slooten et al. 1998) are involved in the regulation of apoptosis. The role of apoptosis in oncogenesis is currently being studied intensively in breast cancer. This short review summarises the main findings of the recent studies that relate apoptosis to other pathological parameters and cellular features in human breast cancer.

Methodological aspects
Apoptosis can be detected in breast cancer by counting the apoptotic cells in conventional histopathological sections (Lipponen et al. 1994) or by using special staining techniques, which are based on in situ labelling of the fragmented DNA (terminal deoxynucleotidyl transferase-mediated UTP nick end-label (TUNEL) staining) (Mustonen et al. 1997, Berardo et al. 1998, Shen et al. 1998, Hassan & Walker 1998). The apoptotic process is often described by means of the apoptotic index (AI), which may refer to the number of apoptotic cells per unit area of tissue or to the percentage of cells undergoing apoptosis.

Abstract
Apoptosis is a frequent phenomenon in breast cancer and it can be detected by light microscopy in conventional histopathological sections or by special staining techniques. The number of apoptotic cells as a percentage of cells present, or the number of apoptotic cells per square millimetre of neoplastic tissue, is usually described as the apoptotic index (AI). In breast cancer, the AI is not related to tumour size, axillary lymph node metastasis or distant metastasis at diagnosis. It is greater in invasive ductal carcinomas than in other histological types. High AI is also related to high histological grade, high nuclear grade, comedo-type necrosis, lack of tubule formation, and dense infiltration of the tumour by lymphocytes. Sex steroid receptor-negative tumours have greater AIs than the sex steroid receptor-positive ones. Aneuploid breast cancers with high S-phase fractions (SPFs) also have high AI values compared with diploid tumours with low SPFs. p53-Positive breast cancers have high AIs, whereas tumours that are Bcl-2 positive have low AIs. The AI shows a strong positive correlation to all direct or indirect indicators of cell proliferation, such as mitotic index and Ki67 immunolabelling. Univariate survival analyses show that a high AI is linked with unfavourable disease outcome in axillary lymph node-negative and -positive breast cancer, but multivariate analyses indicate that AI is not an independent prognostic factor. In conclusion, a high AI is related to malignant cellular features and indicators of invasiveness and cell proliferation in breast cancer.
other analysed parameters can usually be reliably compared. Both of the methods of detection of apoptosis have their characteristic problems. The identification of apoptotic cells in conventional sections may be difficult, especially when the tumour is densely infiltrated by inflammatory cells or when necrosis is present. Special staining techniques may identify cells that contain fragmented DNA as a result of causes not related to apoptotic cell death (Harn et al. 1997). The variation in apoptosis within a tumour is considerable (Ellis et al. 1998) and therefore results based on analysis of a small number of cases must be interpreted critically, and those studies that have analysed a small number of cells may be biased. Intraobserver reproducibility of apoptotic indices is usually high (R=0.88) (Lipponen & Aaltomaa 1994), but interobserver reproducibility is clearly lower (R=0.42) (Zheng & Zhan 1998) and, indeed, has rarely been reported.

**Apoptotic index related to pathological parameters in breast cancer**

**Tumour size**

Three different studies including a total of more than 1000 breast carcinoma cases have analysed the possible relationship between tumour size and AI (Lipponen et al. 1994, Berardo et al. 1998, Zhang et al. 1998). In only one of those studies, which included 126 breast carcinomas, did large tumours (diameter >5 cm) have significantly higher AIs than small tumours (diameter <2 cm). The mean S.E. AI in small tumours (<2 cm) was 6.6(1.4)/mm², compared with 14.9(4.9)/mm² in large (>5 cm) tumours (P=0.008) (Zhang et al. 1998).

**Distant metastasis and metastasis to axillary lymph nodes**

Distant metastasis (M-category) was found to be independent of AI (Lipponen et al. 1994), whereas the findings relating AI to axillary lymph node status are variable. Zhang et al. (1998) reported a significant relationship between high AI and axillary lymph node involvement: the mean (s.e.) AI was 8.4(1.2)/mm² in node-negative tumours, 12.5(2.1)/mm² in tumours with one to three nodes involved, and 14.8(2.1)/mm² in tumours with more than three nodes involved (P=0.01). In an earlier study that included 288 breast cancers and used similar methodology, no such relationship was present (Lipponen et al. 1994). In a large study (n=979) of axillary lymph node-positive breast cancer, a high AI was positively correlated with the number of involved nodes, but the relationship was only of borderline significance (P=0.06) (Berardo et al. 1998). Advanced stage, combining information from tumour size, axillary lymph node status and distant metastasis, has been related to high AI in one study that included 126 invasive breast cancers (P=0.03) (Zhang et al. 1998).

**Histological type and special histological features**

In invasive ductal carcinomas, AIs are usually higher than in the other histological types (papillary, lobular, mucinous), but medullary carcinomas also show high AIs (Lipponen et al. 1994). However, the findings are not consistent, as the AIs reported in some small studies have been about equal in invasive lobular and invasive ductal carcinomas (Mustonen et al. 1997). Tumours that show extensive tubule formation or other histological features linked with high differentiation have low AIs. Those being densely infiltrated by lymphocytes also have high AIs (Lipponen et al. 1994), but the data are not entirely consistent (Grekou et al. 1996). Tumours showing necrosis (Lipponen et al. 1994) and, in particular, tumours that have comedo-type necrosis have high AIs (Harn et al. 1997, Mustonen et al. 1997, Shen et al. 1998). Ductal carcinoma in situ lesions have been found to have higher AIs than do invasive carcinomas (Harn et al. 1997, Mustonen et al. 1997, Shen et al. 1998), the difference in AIs being about twofold (Harn et al. 1997, Shen et al. 1998), although opposite results have been reported (Mustonen et al. 1997); however, Mustonen et al. (1997) also found that low-grade carcinoma in situ had lower AIs than the high-grade lesions.

**Nuclear and histological grades**

All the published reports have established a significant relationship between nuclear grade, histological grade (Bloom & Richardson, 1957) and AI (Lipponen et al. 1994, Mustonen et al. 1997, van Slooten et al. 1998, Zheng & Zhan 1998, Zhang et al. 1998). The AIs in grade 3 tumours are about twice as high as those in grade 1 tumours; grade 2 tumours have AIs between these two extremes (Lipponen et al. 1994). In the largest cohort reported to date, the mean(S.E.) AIs, based on morphological analysis, were 7.9(1.3)/mm², 9.4(1.1)/mm² and 16.8(1.5)/mm² in grade 1, grade 2 and grade 3 tumours respectively (Lipponen et al. 1994).

**Oestrogen receptor and progesterone receptor content**

Data relating AI to sex steroid receptor content are variable. Two large studies (Lipponen et al. 1994, Berardo et al. 1998) that included a total of more than 1000 breast carcinomas have established a significant relationship between sex steroid receptor negativity and high AI. Tumours that express oestrogen receptor (ER) have usually lower AIs than ER-negative tumours, so that the
AI values are about 50% greater in ER-negative tumours (Lipponen et al. 1994, Berardo et al. 1998). The relationship between progesterone receptor (PR) and AI is similar, but weaker, in that PR-positive tumours show about 30% lower AI values than do PR-negative ones (Lipponen et al. 1994, Berardo et al. 1998). In recent studies that included only a small number of cases (Harn et al. 1997, Zhang et al. 1998), the relationship between AI and sex steroid receptors did not reach the level of statistical significance (P=0.1-0.2), although a trend was present. It may be concluded, on the basis of all the available data, that sex steroid receptor content and AI show a statistically significant inverse relationship.

DNA ploidy and S-phase fraction

All the published reports have established a positive correlation between apoptosis and aneuploidy (Lipponen et al. 1994, Berardo et al. 1998). The amount of apoptosis by morphological criteria is about 30% greater (P=0.04) in aneuploid tumours than in diploid ones (Lipponen et al. 1994). In a large study that included 979 axillary lymph node-positive breast carcinomas, 35% of diploid tumours and 51% of aneuploid tumours had AIs greater than 1% (Berardo et al. 1998). The S-phase percentage shows a significant positive correlation with AI (Lipponen et al. 1994, Berardo et al. 1998) and this relationship is particularly clear in aneuploid tumours (Samoszuk et al. 1996). The mean(S.E.) AI in tumours with S-phase <7% was 8.0(0.9)/mm², compared with 14.4(1.2)/mm² in tumours with S-phase >7% (P=0.01) (Lipponen et al. 1994). Thirty-nine percent of tumours in the low S-phase group had AIs greater than 1%, compared with 53% of those in a high S-phase group (Berardo et al. 1998).

Mitotic index and Ki67 expression

Mitosis and apoptosis are closely interrelated (Lipponen et al. 1994, Lipponen & Aaltomaa 1994) and, according to logistic regression analysis, mitotic index is the most important determinant of AI (Lipponen et al. 1994). The mitotic index and apoptotic index are almost equal in breast cancer, or the AI may be even greater than the mitotic index (Staunton & Gaffney 1995). In breast cancer, mitotic index and AI have been found to show a highly significant positive correlation (Lipponen et al. 1994, van Slooten et al. 1998, Zheng & Zhan 1998). The correlation coefficient between AI and mitotic index is 0.767 (Zheng & Zhan 1998). In tumours with a mitotic index less than 10, the mean(S.E.) AI was 4.5(0.5)/mm², compared with 15.4(1.1)/mm² in tumours with a mitotic index greater than 10/mm² (P<0.0001) (Lipponen et al. 1994). Other indicators of cell proliferation, such as Ki67 expression (Pietiläinen et al. 1996, Ellis et al. 1998, van Slooten et al. 1998), also have been found to be highly significantly related to AI, in studies based on image analysis (Pietiläinen et al. 1996) or conventional light microscopy (Ellis et al. 1998, van Slooten et al. 1998). In a series of 49 breast carcinomas, 84% of tumours in which the proportion of Ki67-positive cells was less than 20% also had an AI below the median value (0.77%); 61% of tumours in which there was more than 20% of Ki67-positive cells had AIs greater than that median value (P=0.001) (van Slooten et al. 1998).

Overexpression of p53

Breast tumours that overexpress p53 protein are also rapidly proliferating (Pietiläinen et al. 1995), and p53 overexpression is also related to a high AI (Lipponen et al. 1994, Pietiläinen et al. 1995, Mustonen et al. 1997, Berardo et al. 1998, Zhang et al. 1998). The mean(S.E.) AI was 1.31(1.46)% in p53-negative tumours, and 2.34(1.60)% in p53-positive tumours (P=0.04) in a series of 49 breast cancers (van Slooten et al. 1998). Lipponen et al. (1994) reported the AI to be 7.5(1.4)/mm² in p53-negative tumours and to be twice as great in p53-positive tumours (P=0.004), and Berardo et al. (1998) reported that AI was greater than 1% in 52% of p53-positive tumours and in 39% of p53-negative tumours (P<0.0001) in a series of 979 breast carcinomas. However, in a series of 71 breast carcinomas, Zheng & Zhan (1998) found no significant relationship between AI and overexpression of p53.

Bcl-2 expression

Bcl-2 is expressed in well-differentiated sex steroid receptor-positive breast cancers (Lipponen et al. 1995). In theory, Bcl-2 should inhibit apoptosis (Hockenbery 1992), and the relationships in breast cancer support this (Lipponen et al. 1995, Mustonen et al. 1997, Ellis et al. 1998, van Slooten et al. 1998, Zhang et al. 1998). The AI is about 30% lower in Bcl-2-positive breast cancers than in Bcl-2-negative tumours (Lipponen et al. 1994); the staining intensity for Bcl-2 also shows an inverse relationship with AI (Lipponen et al. 1995). The correlation coefficient between AI and expression of Bcl-2 has been reported to be -0.33 (P=0.04) (Ellis et al. 1998). Van Slooten et al. (1998) reported that 80% of Bcl-2-positive breast tumours had an AI below the median value (0.77%); it was greater than the median in 80% of Bcl-2-negative tumours (P<0.001). In one large study that included 979 axillary lymph node-positive tumours, the relationship between apoptosis and Bcl-2 expression was not statistically significant (P=0.1), although a trend was present (Berardo et al. 1998). However, the investigators used categorical analysis (negative or positive) for Bcl-2 expression and apoptosis; it is my belief that these
Lipponen: Apoptosis in breast cancer

methodological factors may explain this unexpected finding.

Survival
Three different studies have analysed the prognostic significance of AI. Berardo et al. (1998) reported that increased apoptosis was related to a decreased probability of survival in 979 axillary lymph node-positive breast carcinomas, with a borderline significance (P=0.06). Lipponen et al. (1994) found a statistically significant difference in survival from breast cancer (n=288), depending on the AI value (cut-off point for AI was 10/mm²). The difference in survival at 10 years in axillary lymph node-negative tumours was 10% (P=0.035); in axillary lymph node-positive tumours it was 35% (P=0.008). Zhang et al. (1998) reported a 30% difference in survival at 5 years (P<0.001) in 126 patients with breast carcinoma (cut-off point for AI 11/mm²). None of these three studies (Lipponen et al. 1994, Berardo et al. 1998, Zhang et al. 1998) found independent prognostic value for AI in multivariate analysis of the available prognostic factors.

References
Koskinen PJ & Alitalo K 1993 Role of myc amplification and over-expression in cell growth, differentiation and death. Seminars in Cancer Biology 4 3-12.