

p27^{Kip1} as a prognostic factor in breast cancer: a systematic review and meta-analysis

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Abstract

The aim of this study was to comprehensively evaluate *via* a meta-analysis the association between p27 expression and clinical outcome in breast cancer patients. We conducted a meta-analysis of 20 studies ($n = 6463$ patients) that evaluated the correlation between p27 expression and indicators of breast cancer clinical outcome, including overall survival (OS), disease-free survival (DFS) and relapse-free survival (RFS). Data pooling was performed by RevMan 4.2. A total of 60% (9 of 15) of the studies showed a significant association between p27 high expression and OS, whereas 25% (2 of 8) and 60% (3 of 5) studies demonstrated a correlation between p27 high expression and DFS and RFS, respectively. The relative risks (RRs) were 1.34 (1.26–1.42) for OS ($P < 0.00001$), 1.27 (1.10–1.47) for DFS ($P = 0.001$) and 1.49 (0.92–2.42) for RFS ($P = 0.10$). In lymph node-negative breast cancer patients, the RRs for OS and RFS were 1.84 (1.30–2.59; $P = 0.0005$) and 1.30 (0.20–8.50; $P = 0.78$), respectively. In lymph node-positive breast cancer patients, the RRs for OS and RFS were 2.99 (1.77–5.07; $P < 0.0001$) and 1.49 (0.80–2.77; $P = 0.21$), respectively. This meta-analysis indicates that reduced p27 is an independent prognostic factor for poor overall and disease-free cancer survival.

Keywords: p27 • breast cancer • prognosis • meta-analysis

Introduction

Breast cancer remains a leading cause of female morbidity and mortality worldwide [1]. Several prognostic indicators for this cancer, such as tumour size, lymph node status, histological grade and type, vascular invasion and oestrogen receptor status, have been demonstrated [2]. There is, nevertheless, a considerable

need for reliable prognostic markers to assist clinicians in the management of this cancer [3].

A decade of research on p27 and breast cancer has not improved our ability to draw conclusions relevant to the clinical management of patients with this cancer. Several breast cancer studies have demonstrated that low levels or loss of p27 expression is a significant predictor of reduced survival, which is related to tumour progression and prognosis. Those studies showed that in particular subgroups of breast cancer patients, reduced p27 expression identifies patients with poor outcome independent of other prognostic markers [4–6]. A number of other studies suggested a relationship between p27 and outcome but fell short of statistical significance on multivariate analysis [7–9]. Thus, reports of the prognostic value of p27 seemed to be conflicting. As a result, confirmation by independent groups working on different series of cases is still needed before p27 can be accepted as a

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clinically relevant prognostic marker for breast cancer. A meta-analysis confirming the independent prognostic value of p27 would support further prospective analyses of p27 in the context of clinical trials in which patients received uniform treatment.

To investigate whether p27 is indeed a prognostic factor in breast cancer, we conducted a systematic review of the published literature. In an attempt to review those data quantitatively, we used a meta-analysis to gain insights into whether p27 could provide useful guidance in the management of breast cancer.

Materials and methods

Search strategy and selection criteria

We performed this meta-analysis according to a predetermined written protocol of our group. To be eligible for inclusion in our meta-analysis, studies had to be English-language published studies dealing with histopathologically confirmed primary breast cancer without distant metastases at the time of study inclusion. Studies published between January 1997 and July 2007 were the primary data source for PubMed database and EMBASE searches with the following simultaneously used key words: breast cancer, breast carcinoma, breast tumour, p27, p27^{Kip1} and prognosis. The last query was updated on September 3, 2007. We also searched the reference lists of all selected publications.

Data extraction and handling

Data were extracted in an Access database by two investigators (R.X. and J.B.) trained to interpret information to ensure homogeneity in data gathering and entry. Reviews, non-original articles and studies on breast cancer cell lines and animal models were excluded from our review. To assess the effect of subjectivity and potential systematic biases on data gathering, these investigators extracted data from eligible studies independently and reached consensus on all items. Complete concordance was reached for all main variables assessed in this analysis. To avoid duplicate data, we identified articles that included the same cohort of patients by reviewing inter-study similarities in the country in which the studies were done, investigators in the studies, source of patients, recruitment period and inclusion criteria. When the same investigators reported results obtained on the same cohort of patients in several publications, only the largest series were included in the analysis. Duplicate reports were included in the specific analyses only if they applied different antibodies, different immunoreactivity cut-offs or conducted different subgroup analyses. A cohort of patients was not included more than once in the same analysis. Publication bias was examined by the Begg's test and Egger's test.

Statistical analysis

Analyses were done with Revman version 4.2 review manager software. Results were regarded as significant when the statistical test comparing relative risks (RRs) between the groups with low and high levels of p27 expression had a *P*-value < 0.05. The same threshold was adopted for the

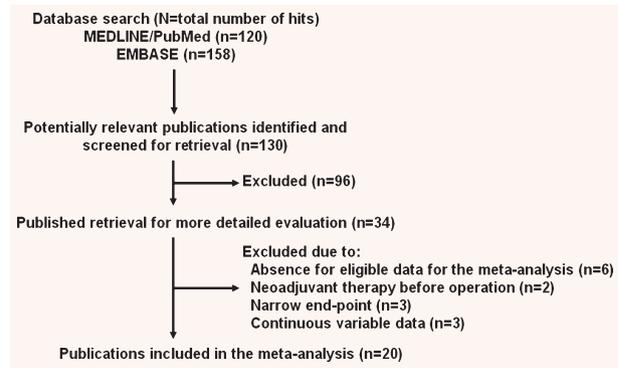


Fig. 1 Methodological flow chart of the systematic review.

multivariate analysis. A study was termed 'positive' or conclusive when low p27 expression predicted poorer survival and was considered 'negative' or inconclusive when low p27 expression did not predict poorer survival or predicted better survival. For the quantitative aggregation of survival results, we measured the impact of p27 expression on survival by estimating the RR between the low- and high-p27-expression groups. For each trial, this RR was estimated by a method depending on the data provided in the publication. The simplest method consisted of the direct collection of RR, hazard ratio and the 95% confidence interval (CI) from the original article. However, not all studies showed censored cases on the Kaplan–Meier curve; if those data were not available, we looked at the total numbers of events and the numbers of patients at risk in each group to determine the RR estimate. When data were only available as graphic survival plots, calculations were done only if the number of steps on the curves equalled the number of events given in the publication, assuming that the rate of censored patients was constant during study follow-up [10]. Considering the many sources of heterogeneity among studies and consequently among their individual RR estimates, we calculated the overall RR by using a random-effect model (Der Simonian and Laird's method). By convention, an observed RR > 1 implied a worse prognosis in the low-p27-expression group. In a meta-analysis of published data, summary RR were estimated by calculating the weighted average of the study-specific log RRs, with weights proportional to the inverse of the variances of the study-specific log RR estimates.

Results

The results of the search strategy for studies are summarized in Fig. 1. The bibliographies of any papers thus identified were also hand searched. Thirty-four relevant articles were initially found, of which 14 were determined to be ineligible for our analysis because of overlapped data from the same study group, absence of eligible data for the meta-analysis, continuous variables analysis, administration of preoperative neoadjuvant therapy or because the study findings were based on cell lines (Table 1) [9, 11–23]. The remaining 20 published studies, which represented a total population of 6463 patients, were included in our analysis (Table 2)

Table 1 Studies excluded from the present meta-analysis

Author	Year	n	Analysis result	Reasons for exclusion	Refs.
Chu	1999	169	OS:S	Continuous variable data	[11]
Reed	1999	77	RFS:NS	Continuous variable data	[9]
Leong	2000	148	RFS:NS, OS:NS	RR cannot be calculated as data absent	[12]
Chappuis	2000	202	DDFS:S	DDFS beyond our research; RR cannot be calculated as data absent	[13]
Leivonen	2001	197	5-year BCSS:S 10-year BCSS:NS	BCSS beyond our research; RR cannot be calculated as data absent	[14]
Spataro	2003	461	DFS:NS	Neoadjuvant therapy before operation	[15]
Barnes	2003	830	OS:NS	Continuous variable data	[16]
Esteva	2003	220	DFS:NS, OS:NS	RR cannot be calculated as data absent	[17]
McCallum	2004	148	S	RR cannot be calculated as data absent	[18]
Chappuis	2005	292	BCSS:S	BCSS beyond our research; RR cannot be calculated as data absent	[19]
Traub	2006	338	DFS:S, DFS _{N-} :S, OS:S	RR cannot be calculated as data absent	[20]
Kamel	2006	45	DFS:S	RR cannot be calculated as data absent	[21]
Gonzalez-Angulo	2006	58	OS:S, DFS:S	Neoadjuvant therapy before operation	[22]
Millar	2007	60	NS	The research end-point is narrow	[23]

DFS: disease-free survival; RFS: relapse-free survival; OS: overall survival; DDFS: distant disease-free survival; BCSS: breast cancer specific survival; RR: relative risk; _{N-}: negative lymph node; _{N+}: positive lymph node; S: significance and NS: no significance.

[4–8, 24–38]. Pertinent characteristics and findings for the 18 eligible studies are listed in Table 2.

The ages of patients ranged from 20 to 92 years, and the time to follow-up ranged from 11 to 258 months. Most patients had clinical stage I or III disease. In several studies, many patients from the initial series were excluded because of insufficient histological material, insufficient clinical data or other reasons which may lead to a potential recruitment bias. In 14 of the 20 studies, the data were obtained directly from the original articles. In the other six studies, RRs were estimated by calculating the survival curve. RRs obtained from multivariate analyses were more accurate than RRs obtained from univariate analyses (if both were available for the studies) because intermixed factors were included in the multivariate analyses.

Our meta-analysis included only those studies in which estimates (hazard ratio and 95% CI) were derived from the Cox regression method. The included 20 published studies investigated multiple factors of breast cancer prognosis, such as p27 expression level, lymph node metastasis, tumour stage, tumour size, hormone receptor expression level, *CerbB-2* expression level, p53 abnormal expression and cyclin E expression level. Our multivariate analyses of these studies found significant associations between p27 expression changes and clinical outcome. Among 15 studies that investigated the association of p27 levels with overall survival (OS), 9 reported a significant correlation between them, which indicated that low expression of p27 appeared to be an

independent prognostic factor. Two of eight studies that investigated the association of p27 expression with disease-free survival (DFS) reported a statistical significance, and three of five studies that analysed association of p27 expression with relapse-free survival (RFS) reported that low p27 expression was a disadvantage factor for RFS.

Figure 2A shows the RRs for OS of the 20 studies included in our meta-analysis. Heterogeneity was significant ($P = 0.02$), so we calculated the overall RR by using a random-effect model (Der Simonian and Laird's method). The overall RR for OS was 1.34 (1.26–1.42; $P < 0.00001$).

Nodal status is one of the most potent prognostic factors of breast cancer, and an interesting observation concerns the different associations of p27 with prognosis among patient groups with different nodal status [24]. To evaluate whether nodal status affects the correlation of p27 with prognosis, we further divided the whole study population into two subgroups according to lymph node status: node-negative and node-positive disease. Because neither subgroup showed significant heterogeneities, we applied a fixed-effect model (Mantel–Haenszel) to assess the RRs for various studies. In lymph node-negative and node-positive subgroups, the RRs for OS were 1.84 (1.30–2.59; $P = 0.0005$) and 2.99 (1.77–5.07; $P < 0.0001$), respectively (Fig. 2A).

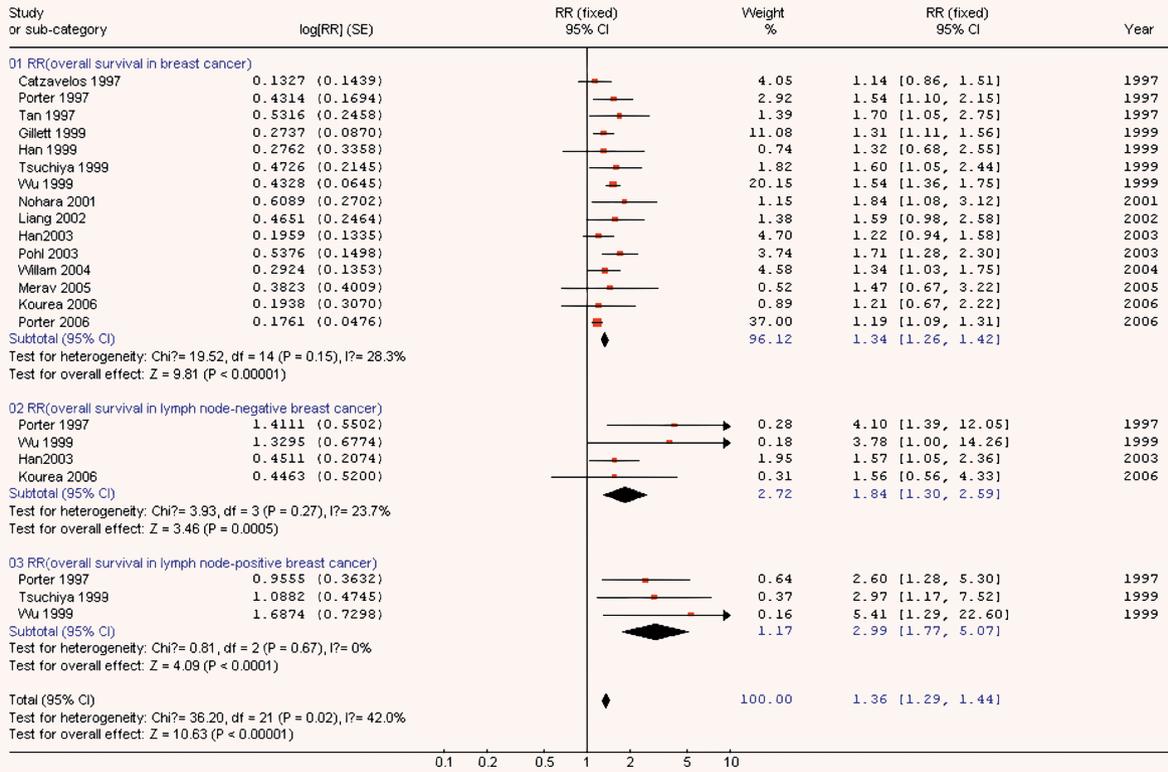
Among all the studies in our review, the population sample of Porter and coworkers [36] which included a total of 2031 cases and accounted for 31.42% of all 6463 cases combined from the

Table 2 Studies that examined p27 protein expression and were included in the present meta-analysis

Author (year – country)	n	Median follow-up(m)	Clinical stage	Cut-off	Antibody	RR estimation	Analysis result	Ref.
Catzavelos (1997 – Canada)	168	NA	I–III	>50%	MP27 transduction	Given by author	DFS:S, OS:NS	4
Tan (1997 – America)	202	65.9	I–III	≥50%	MP27 Transduction	Given by author	OS:S	5
Porter (1997 – America)	246	62.4	NA	>50%	Polyclonal P27	Given by author	OS:S, OSN-:S	6
Tsuchiya (1999 – Japan)	102	NA	II–III	≥50%	MP27 transduction	Survival curves	DFSN+:S, OSN+:S	24
Gillett (1999 – England)	512	198	NA	>5	MP27, 53G8	Survival curves	RFS:S, OS:S	7
Han (1999 – Korea)	68	46	I–III	≥20%	MP27, G173–524 Pharmingen	Survival curves	RFS:S, OS:NS	25
Wu (1999 – China)	181	60	I–III	>50%	MP27 Pharmingen	Survival curves	RFS:S, OS:S	26
Barbareschi (2000 – Italy)	512	NA	I–III	>50%	K2505 transduction	Survival curves	RFS:NS, RFSN-:S, RFSN+:NS	27
Volpi (2000 – Italy)	286	74	I–III	>60%	MP27 transduction	Survival curves	RFSN-:NS	8
Lau (2001 – America)	147	NA	I–III	>50%	MP27 transduction	Given by author	DFS:S \times , NS ϕ	28
Nohara (2001 – Japan)	216	56	I–III	≥62.4%	Clone 57 transduction	Given by author	OS:S	29
Liang (2002 – Canada)	128	NA	I–III	>50%	MP27 transduction	Reported in text	DFS:S(NA), OS:S	30
Pohl (2003 – Austria)	512	156	I–II	≥50%	Clone 57 transduction	Given by author	RFS:S, OS:S	31
Han (2003 – Korea)	175	NA	0–II	≥50%	MP27 NeoMarkers	Given by author	OSN-:NS	32
Foulkes (2006 – America)	247	95.2	NA	>50%	MP27 Transduction	Given by author	OS:S	33
Schöndorf (2004 – Germany)	282	87	I–III	NA	Clone1B4 Novocastra	Given by author	DFS:NS	34
Slotky (2005 – Israel)	50	72	I–III	≥50%	NA	Reported in text	DFS:S(NA), OS:NS	35
Porter (2006 – America)	2031	84	I–III	≥6	Polyclonal P27	Given by author	DFS:S, OS:S	36
Kourea (2006 – Greece)	170	99	I–II	>50%	MP27	Given by author	DFS:NS, OS:NS	37
Tsutsui (2006 – Japan)	228	80.4	I–III	>50%	MP27 Novocastra	Given by author	DFS:S	38

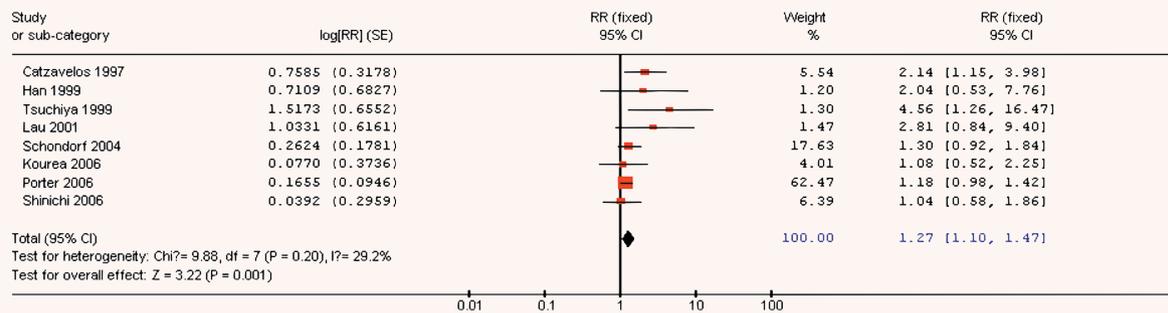
DFS: disease-free Survival; RFS: relapse-free survival; OS: overall survival; RR: relative risk; N-: negative lymph node; N+: positive lymph node; S: significance; NS: no significance; \times : Univariate analysis; ϕ : Multivariate analysis; m: month and NA: not available.

Review: Prognostic significance of p27 expression in women with breast cancer
 Comparison: 01 low p27 VS high p27
 Outcome: 02 01RR(overall survival in breast cancer patients)



A

Review: Prognostic significance of p27 expression in women with breast cancer
 Comparison: 02 low p27 VS high p27
 Outcome: 01 RR(disease-free survival in breast cancer patients)



B

Fig. 2 Forest plots of risk ratios (RRs) for survival in breast cancer patients. (A) RR for overall survival (OS); (B) RR for disease-free survival (DFS) and (C) RR for relapse-free survival (RFS). William 2004, Merav 2005, and Shinichi 2006 refer to Foulkes 2004 [Ref. 33], Slotky 2005 [Ref. 35], and Tsutsui 2006 [Ref. 38] in Table 2, respectively.

20 selected articles, was much larger than the samples from any of the other studies. We attempted to assess the change in RR with sample size reduction by excluding this large study. The RR for OS increased to 2.29 (1.92–2.74; $P < 0.00001$). Publication

bias was not found to be significant ($P = 0.669$ for the Begg's test and $P = 0.169$ for Egger's test).

Eight articles were selected for the meta-analysis of DFS. Because no significant inter-study heterogeneity was found

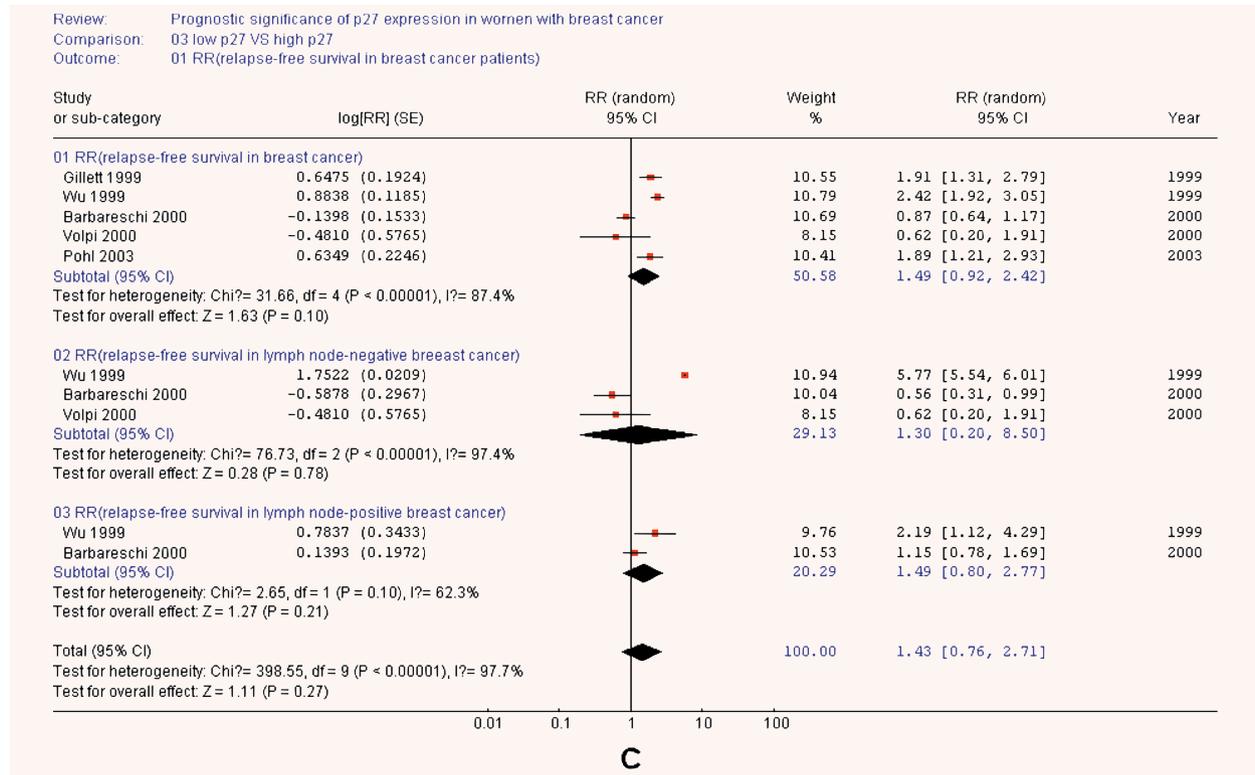


Fig. 2 Continued.

($P = 0.20$), we applied the fixed-effect model. The synthesis RR was 1.27 (1.10–1.47; $P = 0.001$; Fig. 2B). When the study by Porter and coworkers [36] was excluded, the RR for DFS was 1.44 (1.14–1.83; $P = 0.003$). Still, no publication bias was detected in this analysis ($P = 0.108$ for Begg's test and $P = 0.06$ for Egger's test).

Figure 2C shows the RR for RFS in breast cancer by p27 expression and lymph node status. Five studies were included in a separate meta-analysis for RFS. Because inter-study heterogeneity was significant ($P < 0.00001$), we applied the random-effect model, and the calculated RR was 1.49 (0.92–2.42; $P = 0.10$). After stratification according to lymph node status, the overall RR of lymph node-negative groups was 1.30 (0.20–8.50; $P = 0.78$) where the random-effect model was applied because of significant heterogeneity ($P < 0.00001$). Because there was no heterogeneity among lymph node-positive groups ($P = 0.08$), fixed-effect mode analysis was applied and the synthesis RR was 1.49 (0.80, 2.77; $P = 0.21$). Again, no publication bias was detected ($P = 0.462$ for Begg's test and $P = 0.523$ for Egger's test).

Discussion

Conventional prognostic factors such as tumour stage, grade, size and multifocality do not accurately predict the clinical outcome in

many patients with breast cancer [39]; thus, the search for better prognostic indicators and new predictors is of utmost importance [40]. Clinically, it is crucial to identify those patients who would benefit from adjuvant chemotherapy by appropriate prognostic markers. Many studies have indicated that changes in p27 expression in breast cancer can be used to predict survival and response to chemotherapy [4–10], and many observational studies have concluded that p27 is a prognostic factor in breast cancer [4–6], whereas other studies suggested a relationship between p27 and outcome but fell short of statistical significance [7–9]. To determine whether p27 can serve as a prognostic factor in breast cancer, we undertook a systematic review of the literature with a meta-analysis.

In the articles reviewed in our meta-analysis, we found that the prognostic significance of p27 varied substantially among studies. Catzavetos and colleagues reported that low (<50%) levels of p27 correlated with decreased DFS but not with OS in a multivariate analysis [4]. Tan and colleagues reported that T1a and T1b breast carcinoma lacking p27 expression were associated with lower OS in a multivariate analysis [5]. Using univariate and multivariate analyses, Wu and coworkers noted a prognostic significance of p27 for OS and DFS in both node-negative and node-positive breast cancer [26]. In contrast, Barbareschi and coworkers reported that p27 expression did not predict outcome in their whole series of cases but that high p27 expression indicated a poor prognosis even in node-negative cases [27].

Our meta-analysis results showed that reduced levels of p27 appear to be an independent prognostic factor for breast cancer as indicated by OS (Fig. 2A) and DFS (Fig. 2B) but not by RFS (Fig. 2C). Ideally, a strong prognostic role needs to be supported by high RR with low *P*-value. Some researchers even suggested that prognostic factor that has an RR < 2 could be of limited practical use [41, 42]. Accordingly, our statistical results should be interpreted with caution considering the relatively low RRs for OS and DFS in the whole population. However, this does not necessarily negate the prognostic role of p27 in breast cancer patients. It is noteworthy that in most clinical outcome evaluation of studies of HER-2 and ER, two of the most clinically important molecular prognostic markers for breast cancer, the RR do not reach 2. Thus, despite its relatively low RR, low p27 may prove to be a useful marker in clinical practice to assess the outcome of breast cancer patients. Nevertheless, this needs to be substantiated by further confirmation in prospective studies.

Our statistical results indicated that p27 expression in breast cancer is strongly predictive for OS, especially in node-positive patients, but weakly predictive for DFS. A possible explanation is that studies that evaluate OS usually have patient groups with longer follow-up, and include more patients in order to obtain statistical results on patient survival. Thus, OS studies tend to include stronger data with longer patient follow-up than those reporting DFS, yielding stronger data.

The wide heterogeneity in results among the studies could have been caused by differences in several characteristics of their designs, including population sample size, homogeneity of tumour stage, geographic area where the research was done, year of publication, length of recruitment period, inclusion criteria, previous treatment, sample storage, primary antibody and dilution, antigen-retrieval technique, cut-off value, end-point definition, follow-up period, statistical strategy and adjustment for cofactors [43]. For example, different antibodies used and different cut-off values (Table 2) were used in the studies included in this meta-analysis. Among the eligible studies, three independent studies (*n* = 576) included only patients with lymph node-negative disease [32, 33, 37], and the length of median follow-up in the studies varied substantially (from 72.3 months in the studies of Tan and colleagues to 21.5 years in the reports of Gillett and coworkers [5, 7]). Among all these characteristics, lymph node status remains the most important; differences in lymph node status usually produce heterogeneous results. We therefore investigated whether lymph node-positive and -negative status affected the results of survival analyses using stratification and multivariate unconditional logistic regression models for every end-point. The dependent variable was a *P*-value ≤ 0.05.

Moreover, subjectivity in interpretation of the results could have accounted for additional inconsistencies. For instance, variability in both the length of follow-up and the strategies used to detect the events of interest could also hamper comparability among studies, as the risks of recurrence and progression are time dependent and introduce uncertainty into the comprehensiveness of identification of clinical events. Thus, Leivonen and coworkers reported that tissue expression of p27 was a significant

predictor of 5-year but not of 10- or 15-year breast cancer-specific survival [14].

Another important issue that we need to take into account is the type of adjuvant systemic therapy that each patient receives. It is known that chemotherapy and/or hormone therapy can change the outcome in early-stage breast cancer and possibly, in other-stage breast cancers. However, majority of published studies provide a lack of detail regarding patient treatment, and most of the retrospective analyses of p27 are ones in which patients lacked uniform treatment and in which chemotherapy regimens relevant to current therapy were not used. We need to consider whether p27 could always be a promising prognostic indicator regardless of what kind of therapy the patients receive. Because it is almost impossible to evaluate the prognostic role of p27 in never-treated breast cancer (where long-term follow-up without treatment is not practical), we attempted to perform a stratified analysis in subgroups employing different therapeutic strategies. As shown in Fig. 3, the RRs for OS and DFS in breast cancer patients who received adjuvant treatment were 1.58 (1.26–1.97; *P* < 0.0001) and 1.20 (1.00–1.44; *P* = 0.05), respectively. The expression of p27 appeared to be an independent prognostic factor for OS but not for DFS. However, the patient populations we included and the treatments they received were very heterogeneous, which impeded us from conducting further stratified analyses.

We attempted to minimize publication bias by making our literature search as complete as possible; however, we could not take into account the few studies published in abstract form only or in a language other than English. Thus, the risks calculated in our meta-analysis could be overestimated as a result of these biases [44]. Additionally, of the 14 studies excluded (Table 2), a majority (8 out of 14) show a statistical correlation between high p27 and good outcome. Several other studies reporting non-significant associations were excluded from our meta-analysis because they did not have data for hazard ratios and 95% CI, and those exclusions may also have resulted in biases.

Moreover, it is difficult to draw any conclusions when the studies are not conducted prospectively and when not all relevant data are available. For example, we could not obtain original data regarding the therapies each patient received, thus could not be able to conduct further stratified analysis. Further examination of p27 in large prospective series with long-term follow-up will be necessary before definitive conclusions on the prognostic significance of p27 expression can be drawn.

The prognostic role of a specific molecular marker is more powerful when used to help make therapeutic decisions. Many reports have shown that HER-2 is a marker of poor prognosis in breast cancer. However, where it is most clinically useful is as a predictive marker used to guide treatment decisions about whether to use targeted therapy such as trastuzumab. In contrast to HER-2, the oestrogen receptor is associated with good prognosis. Similarly, ER is mainly used as a biomarker to guide treatment decisions about whether hormone therapy is appropriate. Understanding the role of p27 in breast cancer and thus developing corresponding targeted therapy will benefit, at least some subpopulations of, breast cancer patients the most.

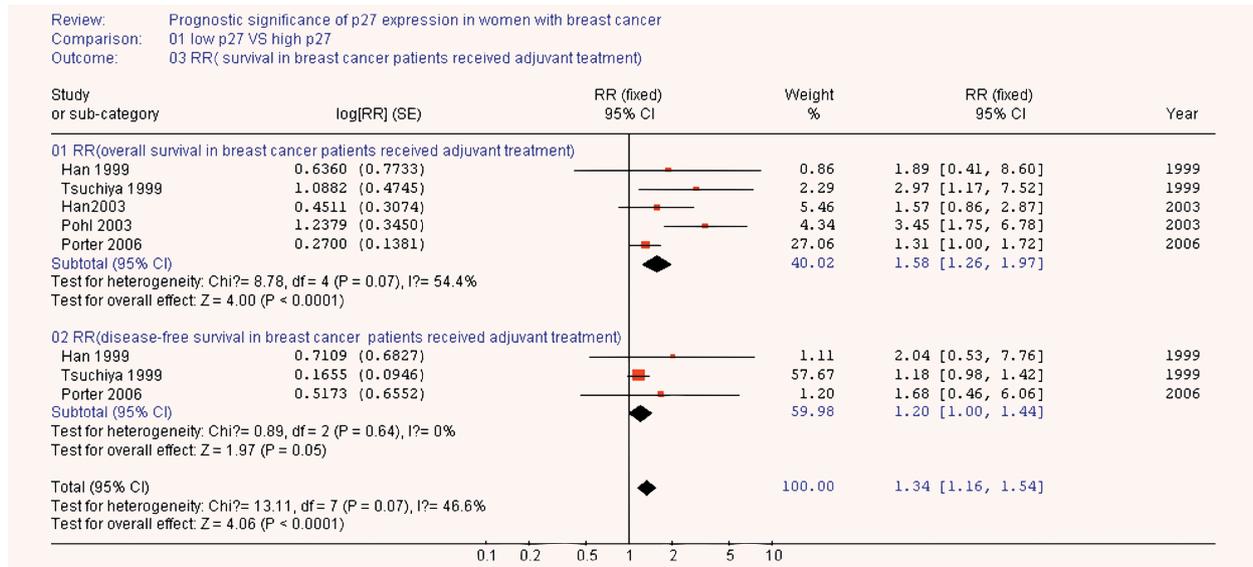


Fig. 3 Forest plots of RRs for OS and DFS in breast cancer patients who received adjuvant treatment.

The mechanism of inactivation of p27 also warrants attention. Functional inactivation of the tumour suppressor p27 in human cancer is due either to loss of expression or to phosphorylation-dependent cytoplasmic sequestration. The action of p27 is impaired in breast and other human cancers through accelerated p27 proteolysis, and sequestration occurs by p27 mislocalization in tumour cell cytoplasm [45]. Although intracellular concentration as well as sub-cellular localization would change the function of p27 in tumour cells [46], most studies that we reviewed (except for two [30, 37]) did not investigate the correlation of cytoplasmic and nuclear expression of p27 with clinical outcome in breast cancer simultaneously. One of the reviewed studies reported that cytoplasmic p27 had previously been correlated with a poor patient prognosis [30] and that further studies were needed to verify that conclusion.

Screening p27 residues identified many potential phosphorylation sites. It has been demonstrated that phosphorylation controls the stability and expression of p27 [47, 48]. However, it is still uncertain whether different phosphorylation statuses of p27 have any clinical prognostic implication in breast cancer. In the selected studies in our review, p27 was only assessed by regular immunohistochemistry using antibodies such as MP27 transduction, K2505 transduction and clone 57 transduction; however, the phosphorylation status of p27 was never considered. Regular immunohistochemistry has been most commonly used to assess p27 expression status because this method can detect changes present in a small proportion of tumour cells, is easy to apply in pathology laboratories, is inexpensive, can be readily performed on many sample specimens in a short time and can be done on formalin-fixed tissues, thus allowing retrospective assessment after long-term follow-up. However, phosphorylated p27 targeting antibodies

need to be used to further assess whether phosphorylation of p27 affects outcome in breast cancer patients in the future.

In view of our findings, we make the following recommendation to future investigators of this topic: conduct large prospective studies with long-term follow-up, give a full description of survival events to allow future calculations, compare survival curves and conduct multivariate regression analysis and assess the prognostic role of p27 in the global population and separately in different nodal status subpopulations. Further studies will be required to understand the role of phosphorylation status of p27 in breast cancer patients, and to understand the importance of nuclear to cytoplasmic ratio of p27 distribution to patient prognosis.

In conclusion, our meta-analysis suggests that high expression of p27 is an independent prognostic factor in breast cancer, as judged by OS and DFS but not RFS. It should be emphasized that these findings need to be interpreted with caution, because formal processes for assessing markers should be followed systematically before they are introduced into clinical practice. However, p27 can now be added rather confidently to the limited list of demonstrated prognostic factors in breast cancer. The ultimate contribution of p27 beyond the classic prognostic factors remains to be determined in further studies with longer follow-up.

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