# Master of Biostatistics

# Workplace Project Portfolio

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## Preface

This workplace project is a single study on outcome of women with invasive breast cancer who presented to Prince of Wales Hospital for treatment. This project is retrospective and exploratory in nature, involving detailed analyses on the relationship between breast cancer tumour receptors and clinical outcomes. The first part of this report has been written and formatted as a scientific manuscript which will be submitted to a medical journal for consideration for publication. A statistical appendix outlines in greater details the methodology employed in the analyses of data and some additional results not presented in the main manuscript.

## Location and Dates

This project was conducted in the Department of Medical Oncology, Prince of Wales Hospital (POWH). The project commenced at January 2008 and completed in February 2009. Data analyses commenced at mid-February 2009.

## Context

The project is an audit on the outcomes of breast cancer patients who received treatment in the Department between 1995 and 2005. Moreover, there has been recent interest within the oncology community in breast cancer classifications using readily available clinical and pathology information. This is because such classification system has prognostic significance which will aid clinicians in treatment decision and counselling of individuals.

## Student Contribution

I am the Research Fellow for the Department. I am the principal investigator for this project. I was involved in the design and conduct of this project. These include drafting the project

protocol (including the statistical analysis plan), submission of protocol for ethics review, retrieving of histology slides for pathology review, data entry into spreadsheets and cleaning of pre-existing data from the Breast Database at POWH. I performed all statistical analysis for this project. I am also the principal author of the manuscript attached for this report. The manuscript will be submitted to a medical journal for consideration of publication.

Prof V Gebski (NHMRC Clinical Trials Centre, University of Sydney) is the main statistical supervisor who provides guidance for the statistical analyses of this project.
Two senior clinicians (Dr C Lewis and Prof M Friedlander) of the Department supervised the conduct of this project.

Reflection of learning process

*Work patterns/planning*

This workplace project has taught me the importance of time and organisation management skills. Regular face-to-face meetings and other forms of communications (emails, faxes, telephones) with the supervising clinicians, data manager and statistician were paramount to answer questions and provide guidance to the direction of this project. I initiated a number of these meetings which were very useful to discuss and address problems as the project progressed.

*Communication with clinicians*

Communicating the statistical results in terms that can be easily understood to the clinicians was most the most challenging aspect of this project. Relating the statistical importance and the clinical importance of the findings of this project are paramount for the knowledge gained from this project to be directly translated to the daily clinical practice.

*Relevant BCA Course*

Survival Analysis (SVA) provides me with an understanding of the survival analysis techniques (semi-parametric proportional hazard modelling, Kaplan Meier estimates of survivorship and Cox regression) and was fundamental in describing the details of the statistical methods necessary to implement the survival endpoints.

*Statistical Issues*

These include the appropriate choice of statistical models to handle time-to-event data containing censored information, appropriateness of categorization of continuous variables, proportional hazard assumptions and appropriate clinical interpretation of the statistical results.

*Ethical Consideration*

As the principle investigator, I was involved in drafting and finalising the ethics application for the Research Ethics Committee of the South Eastern Sydney Area Health Service (Northern Network). This project was conducted with due care and diligence in accordance with the requirements as set out by the Ethics Committee and the project aims and objectives. The confidentiality of the individual patient's information was protected and supervisions by senior clinicians and statistician ensured that the conduct and analysis of this project was undertaken responsibly and professionally.

Acknowledgements

I am grateful to Dr C Lewis for his supervision, advice and active involvement in the conduct of this project. Ms E Choo has assisted me with the cleaning of the Breast Database at

POWH. Prof M Friedlander has provided many constructive comments regarding the analyses and interpretation of the data. Finally, Prof V Gebski has provided invaluable guidance with the statistical analyses.

Student Declaration

I declare this project is evidence of my own work, with direction and assistance provided by my project supervisors. This work has not been previously submitted for academic credit.

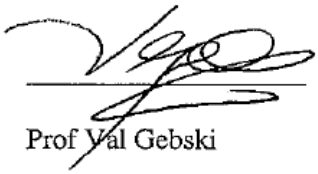
25/6/09

Chee Khoon Lee — Date

SID: 199529303

Declaration by Statistical Project Supervisor

I declare that Chee Khoon Lee has performed the statistical analysis alone on this project under my supervision and was actively involved in the evolution of the project. This work has not been previously submitted for publication or academic credit. To my knowledge, Chee's involvement and effort on this project is highly satisfactory for the requirements of the BCA Workplace Project.



25/06/09

Prof Val Gebski — Date

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Manuscript

Title

Breast Cancer Classification, according to Immunohistochemistry Determination of Oestrogen, Progesterone and HER2 Receptor, has Important Prognostic Value

Abstract

*Background*: Oestrogen (ER), progesterone (PR) and HER2 receptor expression in breast cancer is of prognostic importance and influences treatment recommendations. This study classifies breast cancer (BC) into four subtypes based on receptor status as determined by immunohistochemistry (IHC) and in-situ hybridisation (ISH) analysis on the primary tumour specimens.

*Methods*: This study population comprises 784 women with early stage BC who underwent definitive surgery between 1995 and 2005. In addition to the standard pathology description of the primary cancer specimen, all specimens were analysed for oestrogen (ER), progesterone(PR) and human epidermal growth factor receptor 2 (HER2) expression (IHC) and HER2 ISH analysis if IHC was equivocal. A total of 78% received adjuvant systemic treatments, either chemotherapy, endocrine therapy or both and none received adjuvant trastuzumab. BC was classified into four subsets as follows: Luminal A (ER+ and/or PR+ and HER2-), Luminal B (ER+ and/or PR+ and HER2+), HER2 (ER- and PR- and HER2+) and Basal (ER- and PR- and HER2-).

*Results*: The median follow-up was 38 months (range 0.2 to 139 months). The 3-year overall survival rate for the study group was 86.4%. The 3-year cumulative incidences of death were: Luminal A 3.2% (95% CI 1.8 to 5.8), Luminal B 5.0% (95% CI 1.3 to 18.7), HER2 18.0% (95% 11.5 to 27.7) and Basal 13.6% (95% 5.8 to 29.9). In multivariable analyses with Luminal A as reference group, HER2 (adjusted hazard ratio (AHR) = 4.34, $p<0.0001$) and

Basal (AHR=3.23, p<0.0001) were associated with decreased survival rates. HER2 was also associated with increased distant recurrence rates (AHR = 2.86, p=0.006).
*Conclusion*: The different survival rates among the BC subtypes suggest that the immunohistochemical classification system has prognostic significance and appears to have clinical utility.

## Introduction

Breast cancer is represented by a heterogeneous group of diseases with a wide spectrum of clinical and pathologic features and variable biological behaviour. All breast cancer specimens are routinely tested for expression of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) proteins using immunohistochemistry (IHC), a specialized staining technique. ER, PR and HER2 are receptors located on the membrane of breast cancer cells.

In recent years, gene microarray technology has further classified breast cancers into different subtypes through identification of unique gene expression signatures [1-3]. Amongst the ER positive tumours, Luminal A subtype has the highest expression of ER genes when compared with Luminal B subtype. For the ER negative tumours, the subclass with high expression of HER2 genes is substantially different to the basal subtype, which is characterised by low expression of genes coding for HER2, ER and PR.

Microarray technology remains an expensive research tool and is not yet routinely applicable in laboratory and clinical practice. There has been recent interest in using immunohistochemical determination of ER, PR and HER2 as surrogates of breast cancer

classification. IHC is relatively inexpensive, routinely performed in histopathology laboratories and the results are readily available translated to the clinical practice setting.

Using IHC to define breast cancer subclasses has also been demonstrated to be of prognostic importance. Population-based studies have reported that HER2+/ER- and Basal (ER-, PR- and HER2-) subclasses have the shortest survival[4]. Following breast conservation surgery, Luminal A subtype has the lowest local recurrence rates[5, 6], appears to derive a relatively small benefit from chemotherapy but benefited more from hormonal therapy. In contrast, patients with Luminal B subtype have higher response rates with chemotherapy with improved disease-free survival in the adjuvant setting (preventative treatment administered during early stage of cancer)[7]. Patients with HER2 subtypes have improved disease-free survival when they received adjuvant treatment with trastuzumab and chemotherapy[8, 9]. Basal breast cancers tend to have early distant recurrence and lower survival rates [10-13].

The purpose of this study was to categorise the breast cancer subclasses according to incidence of distant recurrence and survival rates in women with early stage invasive breast cancer following definitive breast surgery. Breast cancer classification is defined using IHC for ER and PR, IHC and cytogenetic techniques for HER2 on the primary tumour.

## Materials and Methods

Between January 1995 and December 2005, a total of 1,256 patients underwent breast surgery at the Prince of Wales Hospital (POWH) were identified. This analysis only included 784 women (62%) with early stage breast cancer with complete information of ER, PR and HER-2 status of the primary tumour. Patients were excluded if they had metastatic breast cancer at diagnosis, carcinoma in situ only without evidence of invasive disease,

inflammatory breast cancer, prior adjuvant treatment with trastuzumab for breast cancer and prior other invasive cancers. This study was approved by the Research Ethics Committee of the South Eastern Sydney Area Health Service (Northern Network).

*Breast Cancer Subgroups Classification*

The breast cancer was classified into four subgroups based on the primary breast tumour: Luminal A (ER+ and/or PR+, and HER2-), Luminal B (ER+ and/or PR+, and HER2+), HER2 (ER- and PR-, and HER2+) and Basal (ER- and PR-, and HER2-). ER and PR status was determined on the basis of IHC staining. Tumours were classified as HER2 positive if they were scored 3+ by IHC or if HER2 gene expression was amplified on in-situ hybridisation testing. For tumours reported as 2+ by IHC or "positive" without any IHC scoring, a second pathology re-evaluation was performed. Cases that remained indeterminate underwent confirmatory cytogenetic testing with either fluorescence in situ hybridization or chromogenic in situ hybridization[14].

*Treatment*

Mastectomy or breast conserving surgery (BCS) was performed in 369 patients (47%) and 407 patients (52%) respectively. A total of 747 patients (95%) underwent axillary nodal surgery to remove suspected lymphatic spread of the cancer.

External beam radiotherapy to the whole breast was administered to 338 patients (83%) who had BCS. The common dose administered was 50Gy in 25 fractions in 2Gy per fraction, administered daily Monday to Friday over 5 consecutive weeks. In addition, as per protocol, the majority of patients received an additional boost of 10 Gy in 5 fractions to the scar.

Decisions regarding the use of adjuvant systemic therapy were based on the recommendation of the Multidisciplinary Breast Clinic, attended by medical, radiation and surgical oncologists, pathologists and allied health personnel. Individualised recommendation for each patient was based on the pathology of the cancer, the patient's general health, functional status and co-morbidities. Of the 784 women, 263 (34%) received endocrine therapy alone, 199 patients (25%) endocrine and chemotherapy and 148 patients (19%) chemotherapy alone. Chemotherapy was administered in 65% and 29% of patients with and without axillary nodal involvement respectively. In general, premenopausal women with involved axillary nodes received adjuvant chemotherapy and tamoxifen if the cancer was hormone receptor positive. Adjuvant tamoxifen or aromatase inhibitors were generally considered for postmenopausal, hormone receptor positive patients with axillary nodal involvement or high risk disease with no axillary nodal involvement.

*Follow-up*

For patients who underwent chemotherapy or radiotherapy, they were generally reviewed in the clinics every 3 months for 2 years, every 6 months for the next two to five years and annually thereafter. During these clinic visits, patients were assessed for disease recurrence. For patients who discontinued follow-up at POWH, attempts were made to obtain information from their general practitioners.

*Study End Points*

The primary endpoint of this study was overall survival. This endpoint was defined as time from histological diagnosis till death or last known alive status. The secondary endpoint was recurrence-free survival defined as time from histological diagnosis till the occurrence of

distant metastasis or death. Patterns of distant metastasis in the breast cancer subgroups were also explored.

The incidence of local recurrence (within the breast) and regional recurrence (draining lymph nodes) was very low (10 (1.3%) and 5 (0.6%) patients respectively) for any meaningful analysis. No death consequent to local or regional recurrences was documented during the study period. These events were not considered in subsequent analyses.

*Statistical Analysis*

The $\chi^2$ test was used to assess the significance of differences in the distribution of baseline categorical characteristics among the four breast cancer subtypes. The overall survival for each breast cancer subtype was estimated using Kaplan-Meier(KM) approach[15] but is expressed as a cumulative incidence curve (1-KM). The proportional hazard model was used to analyse the association between breast cancer subtypes and survival rates. All breast cancer subtypes were compared with Luminal A which was the reference group. For continuous variables (age, tumour size, number of axillary node involvement), the analyses were performed in their original scales. These analyses were also repeated with categorization of the tumour size and number of axillary node involvement based on the TNM classification system[16]. An identical methodology was used for recurrence-free survival. Statistical analyses were performed in Stata 10.1 (StataCorp, College Station, Texas) and ACCorD (Analysis of Censored and Correlated Data, Boffin Software, Sydney). Statistical significance was based on a p-value of less than 0.05 and the analyses were two sided with no adjustment for multiple comparisons.

Results

A descriptive summary of baseline characteristics of patients in this study are outlined in Table 1. In total, 54 patients (7%) were lost to follow-up. The median follow-up time from breast cancer diagnosis till death or last known alive status was 38 months (range 0.2 to 139 months).

Table 1 - Baseline characteristics (n=784)

| Patient & Disease characteristics | | | Number (%) |
|---|---|---|---|
| Age | | <35 | 35 (4) |
| | | 35-45 | 123 (16) |
| | | 45-55 | 222 (28) |
| | | 55-65 | 195 (25) |
| | | 65-75 | 129 (16) |
| | | >75 | 80 (10) |
| Menstrual Status | | Premenopause | 222 (29) |
| | | Peri & postmenopause | 551 (71) |
| Performance Status (PS)* | | 0 | 677 (92) |
| | | 1 | 49 (7) |
| | | 2+ | 11 (1) |
| Tumour size (mm) | | 1-20 (T1) | 425 (54) |
| | | 21-50 (T2) | 316 (40) |
| | | >50 (T3) | 37 (5) |
| Number of axillary nodal involvement | | 0 (N0) | 448 (57) |
| | | 1-3 (N1) | 219 (28) |
| | | 4-9 (N2) | 64 (8) |
| | | >9 (N3) | 52 (7) |
| Histologic Grade | | Well-differentiated | 160 (21) |
| | | Moderately differentiated | 303 (39) |
| | | Poorly differentiated | 308 (40) |
| Histology Subtype | | Ductal carcinoma | 659 (84) |
| | | Lobular carcinoma | 76 (10) |
| | | Other | 49 (6) |
| Lymphovascular invasion | | | 239 (31) |
| Receptor status | | ER+ or PR+ | 603 (77) |
| | | HER2+ | 112 (14) |
| Systemic therapy | No Axillary Node involvement | Endocrine therapy | 226 (50) |
| | | Systemic chemotherapy | 129 (29) |
| | Axillary Node involvement | Endocrine therapy | 236 (70) |
| | | Systemic chemotherapy | 218 (65) |
| Radiotherapy | | | 455 (59) |

*PS 3 and PS 4 have only one patient in each category and hence combined with PS 2 to form PS 2+

*Relationship between Breast Cancer Subtypes and Tumour Characteristics*

Amongst the four breast cancer subtypes, there was a statistical significant difference in the overall distribution of pathologic T stage ($p<0.001$), axillary nodal involvement ($p=0.04$), histologic grade ($p<0.001$), lymphovascular invasion ($p=0.002$) and ductal histology subtype ($p=0.001$) (Table 2).

Table 2 - Comparison of Tumour Characteristics across the Breast Cancer Subgroups

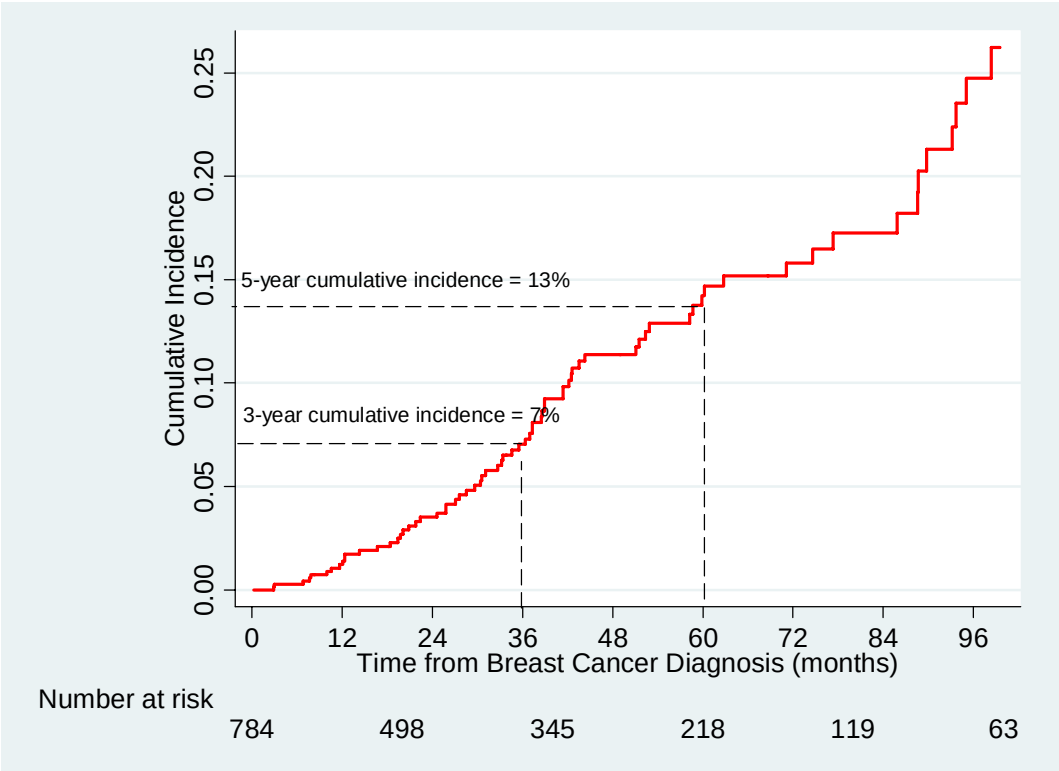
| Baseline characteristics | Luminal A (n=537) | Luminal B (n=66) | Basal (n=135) | HER2 (n=46) | p-value |
|---|---|---|---|---|---|
| Tumour size ≤20mm (T1) | 327 | 34 | 48 | 16 | <0.001 |
| % | 61 | 52 | 36 | 35 | |
| No axillary nodal involvement (N0) | 324 | 31 | 72 | 21 | 0.04 |
| % | 60 | 47 | 53 | 46 | |
| Histologic Grade poorly differentiated | 126 | 33 | 114 | 35 | <0.001 |
| % | 24 | 50 | 87 | 78 | |
| Lymphovascular invasion | 141 | 27 | 51 | 20 | 0.002 |
| % | 27 | 42 | 38 | 43 | |
| Ductal Carcinoma | 431 | 59 | 125 | 44 | 0.001 |
| % | 80 | 89 | 93 | 96 | |

*Overall Survival*

During the period of follow-up, there were 73 deaths. The 3-year and 5-year cumulative incidences of death for all patients were 7% and 13% respectively (Figure 1a). The 3-year cumulative incidences of death for the different breast cancer subgroups were as follows: Luminal A disease 3.2% (95% CI 1.8 to 5.8), Luminal B 5.0% (95% CI 1.3 to 18.7), HER2 18.0% (95% 11.5 to 27.7) and Basal 13.6% (95% 5.8 to 29.9) (Figure 2a).

Figure 1

(a) Cumulative incidence of death in the overall sample




(b) Cumulative incidence of distant metastasis in the overall sample

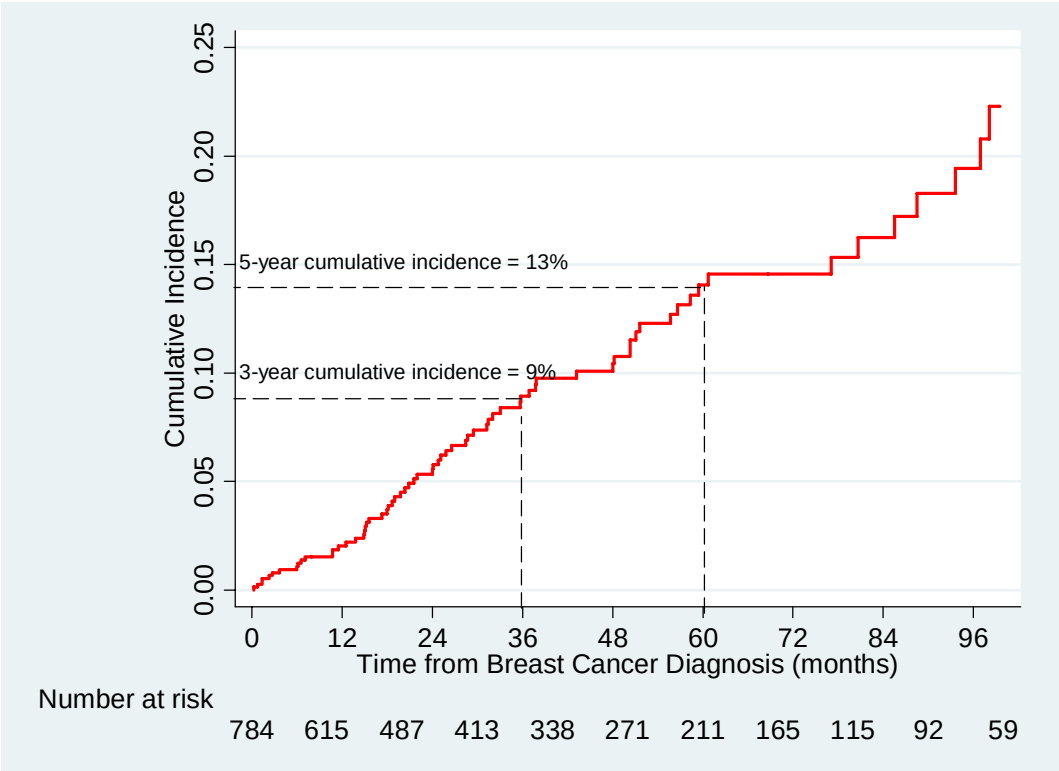
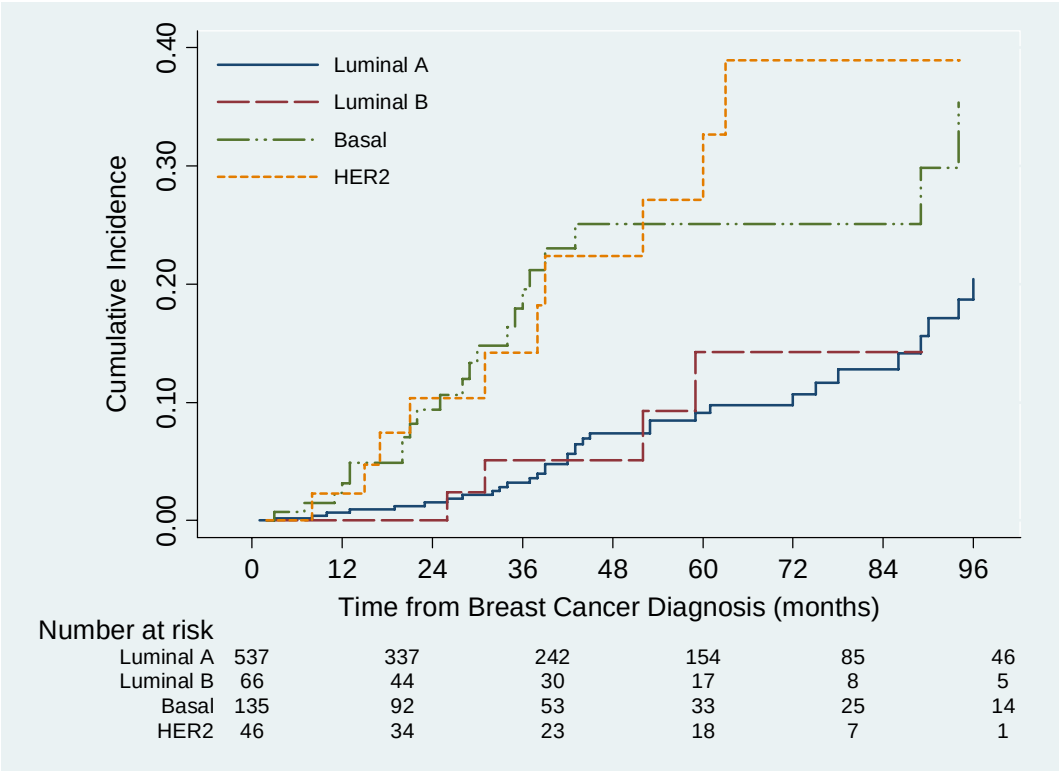
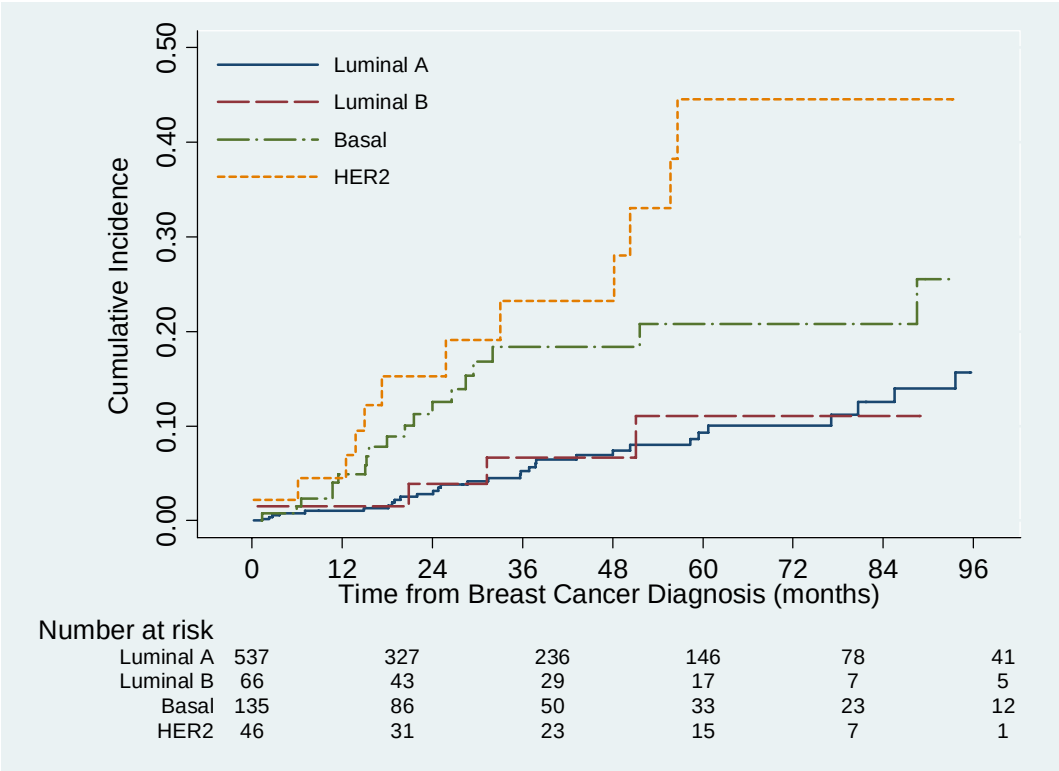


Figure 2

(a) Cumulative incidence of death by breast cancer subgroups




(b) Cumulative incidence of distant metastasis by breast cancer subgroups




HER2, human epidermal growth factor receptor 2

Results of univariable analysis refer to the effect of outcome of each variable when considered singly. On the other hand, multivariable analysis examined the prognostic significance of breast cancer classification after accounting for other baseline parameters.

In a univariable analysis with Luminal A as the reference group, both HER2 (hazard ratio (HR) = 3.11, 95% CI 1.54 to 6.28, p=0.002) and Basal (HR=2.64, 95% CI 1.56 to 4.46, p<0.001) were significantly associated with inferior survival rates. With adjustment of other prognostic factors, the poorer survival rates were still observed for HER2 (adjusted hazard ratio (AHR) = 3.73, 95% CI 1.73 to 8.04, p<0.001) and basal (AHR=1.24, 95% CI 1.01 to 1.52, p=0.001) subgroups (Table 3). Therefore, the interpretation of this result is that for any fixed point in time, patients with HER2 breast cancer are at 3.73 times the risk of dying as compared to patients with Luminal A breast cancer after accounting for other baseline parameters. Patients with basal breast cancer are at 1.52 times the risk of dying as compared to patients with Luminal A breast cancer after accounting for other baseline parameters.

Table 3 - Univariable and Multivariable Analyses of Overall Survival

| Univariable Analysis | | | |
|---|---|---|---|
| Breast cancer subgroups | Hazard Ratio | 95% Confidence Interval | P-value |
| Luminal A | 1.00 | - | - |
| Luminal B | 0.91 | 0.32 to 2.56 | 0.86 |
| Basal | 2.64 | 1.56 to 4.46 | <0.001 |
| HER-2 | 3.11 | 1.54 to 6.28 | 0.002 |

| Multivariable Analysis | | | |
|---|---|---|---|
| Luminal A* | 1.00 | - | - |
| Luminal B* | 0.96 | 0.34 to 2.74 | 0.94 |
| Basal* | 2.95 | 1.70 to 5.13 | <0.001 |
| HER-2* | 3.73 | 1.73 to 8.04 | 0.001 |
| Age (in decades) | 1.24 | 1.01 to 1.52 | 0.04 |
| Tumour size<20mm (T1) | 1.00 | - | - |
| Tumour size 20-49mm (T2) | 1.55 | 0.91 to 2.65 | 0.11 |
| Tumour size ≥50 mm (T3) | 2.92 | 1.10 to 7.74 | 0.03 |
| No axillary node involvement (N0) | 1.00 | - | - |
| Axillary 1-3 lymph node involvement (N1) | 2.49 | 1.37 to 4.53 | 0.003 |
| Axillary ≥ 4 lymph node involvement (N2) | 3.83 | 1.99 to 7.39 | <0.001 |
| Presence of Lymphovascular Invasion | 1.69 | 1.03 to 2.78 | 0.04 |
| Systemic chemotherapy | 0.38 | 0.19 to 0.73 | 0.004 |

* When treated as a factor, breast cancer subgroups remain significant (Wald chunk test: $\chi^2_{(3)}$=19.75, p=0.0002) in the multivariable model.

*Distant Metastases*

During the period of follow-up, 68 patients developed metastatic disease. The 3-year and 5-year cumulative incidences of distant metastasis for all patients were 9% and 13% respectively (Figure 1b). The 3-year cumulative incidences of distant metastasis for the

different breast cancer subgroups were as follows: Luminal A disease 5.1% (95% CI 3.2 to 8.1), Luminal B 6.5% (95% CI 2.1 to 19.4), HER2 16.9% (95% 10.7 to 26.0) and Basal 21.0% (95% 10.9 to 38.2) (Figure 2b).

Amongst the four breast cancer subtypes, there was a statistical significant difference in the overall patterns of liver (p=0.01) and bone metastases (p<0.0001). Liver metastases were predominant in HER2 and Basal subgroups but bone metastases were predominant in the HER2 subgroup. The presence of two or more tissue sites of metastases was predominant in HER2 subgroup (p<0.0001) (Table 4).

Table 4 - Comparison of Metastatic Patterns across the Breast Cancer Subgroups

| Metastatic Pattern | Luminal A | Luminal B | Basal | HER2 | p-value |
|---|---|---|---|---|---|
| Liver metastasis | 10 | 2 | 8 | 4 | 0.01 |
| % | 2 | 3 | 6 | 9 | |
| Lung metastasis | 12 | 2 | 5 | 4 | 0.09 |
| % | 2 | 3 | 4 | 9 | |
| Brain metastasis | 5 | 0 | 3 | 2 | 0.12 |
| % | 1 | 0 | 2 | 4 | |
| Bone metastasis | 23 | 4 | 5 | 9 | <0.0001 |
| % | 4. | 6 | 4 | 20 | |
| ≥ 2 tissue sites of metastasis | 13 | 3 | 9 | 6 | <0.0001 |
| % | 2 | 5 | 7 | 13 | |

In a univariable analysis with Luminal A as the reference group, both HER2 (HR = 3.98, 95% CI 2.05 to 7.72, p<0.001) and basal (HR=2.21, 95% CI 1.26 to 3.89, p=0.006) were significantly associated with an increased rate of distant metastasis. With adjustment of other prognostic factors, only HER2 (AHR = 2.86, 95% CI 1.36 to 6.00, p=0.006) but not basal (AHR=1.47, 95% CI 0.76 to 2.82, p=0.25) was still significantly associated with increased

rate of distant metastasis (Table 5). Therefore, the interpretation of this result is that for any fixed point in time, patients with HER2 breast cancer are at 2.86 times the risk of developing distant metastatic disease as compared to patients with Luminal A breast cancer after accounting for other baseline parameters.

Table 5 - Univariable and Multivariable Analyses of Recurrence-Free Survival

| Univariable Analysis | | | |
|---|---|---|---|
| Breast cancer subgroups | Hazard Ratio | 95% Confidence Interval | P-value |
| Luminal A | 1.00 | - | - |
| Luminal B | 0.97 | 0.34 to 2.73 | 0.95 |
| Basal | 2.21 | 1.26 to 3.89 | 0.006 |
| HER-2 | 3.98 | 2.05 to 7.72 | <0.001 |
| **Multivariable Analysis** | | | |
| Luminal A* | 1.00 | - | - |
| Luminal B* | 0.70 | 0.24 to 2.06 | 0.52 |
| Basal* | 1.47 | 0.76 to 2.82 | 0.25 |
| HER-2* | 2.86 | 1.36 to 6.00 | 0.006 |
| Tumour size<20mm (T1) | 1.00 | - | - |
| Tumour size 20-49mm (T2) | 1.66 | 0.93 to 2.98 | 0.09 |
| Tumour size ≥50 mm (T3) | 2.63 | 1.03 to 6.74 | 0.04 |
| No axillary node involvement (N0) | 1.00 | - | - |
| Axillary 1-3 lymph node involvement (N1) | 1.26 | 0.64 to 2.45 | 0.51 |
| Axillary ≥ 4 lymph node involvement (N2) | 3.31 | 1.73 to 6.31 | <0.001 |
| Lymphovascular invasion | 2.02 | 1.18 to 3.46 | 0.01 |
| Poorly differentiated Histologic Grade | 2.23 | 1.19 to 4.15 | 0.01 |
| Systemic chemotherapy | 0.44 | 0.25 to 0.78 | 0.004 |

* When treated as a factor, breast cancer subgroups remain significant (Wald chunk test: $\chi^2_{(3)}$=8.80, p=0.03) in the multivariable model.

## Discussion

In this study, the 3-year and 5-year overall survival rates were 93% and 87% respectively. Using IHC to determine breast cancer classification we demonstrated that the survival rates of the subclasses of breast cancers vary substantially. We found that HER2 (AHR=4.34) and basal (AHR=3.23) subtypes were associated with decreased survival rates when compared with Luminal A. A similar pattern of variation in breast cancer subgroups was observed for recurrence-free survival where HER2 was associated with an increased recurrence rate when compared with Luminal A.

The findings of decreased survival and increased distant recurrent rates for HER2 subclass was not surprising and is consistent with other studies [4-7, 13] since none of the patient in this study was treated trastuzumab as it was not standard of care for all HER2+ breast cancers during the study period (the drug was not available in Australia). When trastuzumab, a monoclonal antibody, binds the HER2 protein on breast cancer cell surface, tumour growth is inhibited as demonstrated in laboratory models[17-19]. Two large randomised studies confirm the laboratory findings where adjuvant treatment with trastuzumab in HER2+ breast cancer patients demonstrated a relative improvement of disease free survival of 46%[8] and 52%[9] over chemotherapy alone. These studies also demonstrated relative improvement in distant recurrence of 51%[8] and 53%[9]. Similar relative improvement in disease free survival was seen regardless of hormone receptor status[20], indicating that patients from Luminal B and HER2 subclasses derive similar relative benefits with adjuvant trastuzumab.

The 3-year survival and recurrence-free survival rates of 86.4% and 79.0% respectively in patients with basal subclass is also consistent with other studies [4, 6, 7, 10-13]. By definition, the lack of expression of ER, PR and HER2 means that patients in the Basal subclass will not benefit with currently available targeted therapies such as tamoxifen or other endocrine agents or trastuzumab. Preclinical models suggest that basal breast cancer is dependent upon epidermal growth factor receptor (EGRF) pathway for proliferation[21]. However, only limited benefits of treatment with EGFR inhibitor in combination with platinum chemotherapy were reported [22]. The quest for effective treatments with alternative forms of chemotherapy, with or without biologic modifiers, continues and several prospective randomised studies are in progress.

There does not appear to be a clearly demonstrated difference between using either IHC or in situ hybridization (ISH) as a predictor of benefit from trastuzumab therapy[20]. It is also unclear whether the two different methods of determination on breast cancer classification have an impact on clinical outcomes in the untreated population. Our study adopted the approach where tumour blocks with an equivocal IHC result for HER2 (score 2+) underwent confirmatory ISH. We believe that such an approach gave a more accurate determination of HER2 status and was consistent with recommended clinical practice[14].

The major limitation of this study is the assumption that the classification based on IHC is a good approximation of the underlying breast cancer genotypes. For example, only 30% to 50% of Luminal B disease are HER2 positive and hence these cases could be misclassified using IHC[4]. In contrast to microarray classification, information obtained using IHC remains a convenient and clinically applicable method for breast cancer classification. In future, molecular prognostic assays may replace IHC as a more accurate tool to aid treatment

decision. Oncotype DX is an example of such assay that has been developed for classification of women with early stage ER-positive breast cancer into three categories according to their risk of developing distant recurrent disease[23]. Using information from this assay, decision can be made to spare women who are at low risk of unnecessary chemotherapy. However, the predictive ability of this and other assays are still being investigated in ongoing prospective trials.

Another possible limitation of this study is whether patient selection for adjuvant therapy might modify disease outcome. For example, clinical decisions for adjuvant therapy would also include biomedical and social factors. Some of these factors such as age and performance status were recorded and accounted in our statistical models. On the other hand, information on other factors such as social circumstances, patient's attitude and compliance to treatment were not available. The influence of these social factors on outcomes would not be adequately modelled in this study.

The results of this study have a number of implications. Firstly, it will impact on counselling of individual patients with a newly diagnosed early stage breast cancer regarding risk of relapse and prognosis. Secondly, IHC of ER, PR and HER2 receptors remains a convenient breast cancer classification tool for prognostication and treatment decisions. Patients with HER2 breast cancers are recommended to receive adjuvant trastuzumab and cytotoxic chemotherapy in view of their poorer prognosis and evidence of improved survival. Basal / triple negative breast cancer is the subclass now with the worst prognosis due to lack of effective treatment agent.

In summary, women who have undergone definitive breast surgery, IHC classification of breast tumours into four distinct subclasses provides valuable prognostic information which can influence treatment decision.

Statistical Appendix

Objective

The association between breast cancer subgroups with survival and recurrence-free survival outcomes are comprehensively examined in this project in women with early stage breast cancer. This is a retrospective, exploratory analysis to investigate the above hypotheses.

The investigators defined four subgroups of breast cancer based on convenient and readily available clinical information regarding the receptors on the cell surface of the breast tumour:

| Breast cancer subgroup | Receptor status of breast tumour |
|---|---|
| Luminal A | ER+ and/or PR+ and HER2- |
| Luminal B | ER+ and/or PR+ and HER2+ |
| HER2 | ER- and PR- and HER2+ |
| Basal | ER- and PR- and HER2- |

Data Description

*Baseline parameters*

Baseline parameters refer to the clinical and histopathological variables that were recorded in the Breast Database of POWH at the time of diagnosis with invasive breast cancer. These variables are as follows:

*Patients' demographics*

| | |
|---|---|
| Age | Measured in years as a continuous variable but rounded up to the nearest integer. |
| Menstrual status | Pre-menopausal versus peri & post-menopausal. |
| Performance status (PS) | This is a physician assessment of the patient's ability to perform activities of daily living. It is measured on a 5-point scale ranging from 0 (good) to 4(bad). |

*Disease factors*

| | |
|---|---|
| Tumour size | Measured in millimetre as a continuous variable rounded up to the nearest integer. |
| Axillary lymph node involvement | Measured as a continuous variable of the number of axillary lymph nodes with cancer cell metastasis. |
| Histologic grade | "Well" and "moderate" versus "poor" histologic differentiation of the cancer |

| | |
|---|---|
| | cells when viewed under the microscope. |
| Lymphovascular invasion | Presence (versus absence) of invasion of the tumour into lymph and blood vessels as seen under the microscope. |
| ER status | Positive (+) versus negative (-) |
| PR status | Positive (+) versus negative (-) |
| HER2 status | Positive (+) versus negative (-) |
| *Treatment factors* | |
| Endocrine treatment | Anti-oestrogen treatment received (versus none) |
| Systemic chemotherapy treatment | Adjuvant chemotherapy received (versus none) |
| Radiotherapy treatment | Adjuvant radiotherapy received (versus none) |

Data Recoding

Age, which was recorded in years, will produce a small effect size and hence recoded to express in decades to facilitate interpretation of results.

Performance Status (PS) 3 and PS 4 have only one patient in each category. The small number makes any interpretation of subsequent analyses difficult. Hence, the patients from PS3 and PS4 have been combined with PS 2 to form a new category known as PS 2+.

"Well" and "moderated" histologic grade are considered clinically as similar entities. In contrast, "poor" histologic grade is considered to be a clinically aggressive disease. Hence, subsequent analyses compares "poor" histologic grade versus "well" and "moderate".

Recategorisation of Continuous variables

Although categorisation of the continuous variables will lead to loss of power and precision[24], continuous variables are most frequently categorised before the analysis in clinical research. It becomes necessary to maintain some consistency in variables identified as a predictor across different studies. Furthermore, physicians are accustomed to some widely accepted categorised factors of continuously measured variables such as the TNM staging criterion for breast cancer [16]. In this project, the TNM criterion has been adopted to recategorise tumour size and axillary lymph node involvement as follows:

T1 Tumour 20 mm or less in greatest dimension

T2 Tumour more than 20 mm but not more than 50 mm in greatest dimension

T3 Tumour more than 50 mm in greatest dimension

N1 Metastasis in 1 to 3 axillary lymph nodes

N2 Metastasis in 4 to 9 axillary lymph nodes

N3 Metastasis in 10 or more axillary lymph nodes

Outcome measures

Duration of overall survival and recurrence-free survival are the two main outcome measures in this analysis.

Overall survival was measured from the date of histological diagnosis of breast cancer to the date of death. Deaths from all causes, which include the breast cancer, adverse treatment-related events or other unrelated causes, were coded as failures. In all other causes (e.g. lost to follow-up, or alive at the conclusion of the study observation period at 31st December 2005), these women were classified as censored at date of last contact.

Recurrence-free survival was measured from the date of histological diagnosis, to first outcome failure, indicated by (i) death from all causes; or (ii) first distant recurrent disease i.e. development of secondary tumour in other organ sites apart from the breast tissues. For the other women (e.g. lost to follow-up, or alive and no distant recurrence at the conclusion of the study observation period at 31st December 2005), these women were classified as censored at date of last contact.

Both survival and recurrence-free survival were estimated using the Kaplan-Meier approach[15] but expressed graphically as one minus Kaplan-Meier estimates (Figures 1 and 2). The one minus Kaplan Meier estimates are identical to the cumulative incidence estimates since there was no failure encountered from competing events such as local recurrence of cancer on the breast or regional recurrence of cancer on breast or lymph nodes [25].

Methods of Data analysis

*Duration of follow-up of the study population*

Of the 784 patients, 73 (9%) were censored observations for survival. The median follow-up duration for these patients was 38 months. This was obtained by estimating the censoring distribution [26]. Here, a "reverse" Kaplan Meier method was implemented where a survival curve was obtained by treating deaths as censored observations, and loss to follow-up observations as failures. This method avoids the problem of grossly underestimating the follow-up times by just simply obtaining the median survival value from the raw data (and ignoring the censoring status).

*Modelling*

The Cox proportional-hazards model [27] was used for multivariable analysis of the time-to-event data. The hazard ratio was calculated taking the antilog of the regression coefficient. The statistical tests were considered to be significant if the p-values were less than 0.05. There was no adjustment for multiple comparisons.

The association between survival time, breast cancer subgroups, and all the baseline parameters were examined singly. After this univariable analyses, the effect of breast cancer subgroups on survival time was then evaluated after adjusting for other significant baseline parameters, which included the demographics and disease characteristics, and treatment intervention. Variables selected for inclusion into the multivariable model were identified, during univariable analyses, based on the significance level of $p \leq 0.20$. Backward stepwise eliminations of the variables were performed. The "best" final models retained only variables with p-value less than 0.05. Similar approaches were adopted in the secondary analysis for recurrence-free survival.

The analyses were repeated, with the variables as identified in the "best" final models, but with categorisation of tumour size and axillary lymph node involvement according to the TNM staging criterion for breast cancer.

*Proportional Hazards Assumption*

The assumptions made in proportional hazards regression are (1) the hazard ratios do not depend on the event times and (2) the hazard ratios associated with different levels of each variable are constant. These proportional hazards assumptions were checked for each variable included in each of the "best" final models. Tests of significance and graphical methods were used to determine whether these assumptions were met.

The Proportional Hazards (PH) Test of significance is based on methods as detailed by Schoenfeld[28]. A p-value less than 0.05 for any variable may indicate a violation of the proportional hazards assumption for that variable.

Schoenfeld residuals are the differences between the observed values of the variables and their conditional expectations at the respective time points. If proportional hazards hold, a plot of Schoenfeld residual versus event time will be centred about zero. By scaling (average-variance standardization), the residuals could be made to have the same distributions while removing the correlations between the variables. Smoothed plots of the scaled Schoenfeld residuals versus event time can give a visual guidance of the effect of the variable as it varies with time.

*Interaction terms*

There was no prior clinical suspicion of interactions between the breast cancer subgroups and baseline parameters. Only statistical interactions of the breast cancer subgroups with baseline parameters were examined during data analyses. The significance of the interaction terms were tested by performing Wald tests by comparing the model with breast cancer subgroups and other significant baseline parameters without the interaction terms and compared with another model with the same variables and the interaction terms. A p-value less than 0.05 may indicate statistically significant interaction and hence the final best model would be revised to include these interaction terms.

*Collinearity diagnostics*

Multi-collinearity occurs when two or more explanatory variables in a Cox multivariable regression are highly correlated. The involved variables will convey similar information about the outcome. Consequently, multicollinearity often results in large point estimate of the coefficient and inflated standard error for the involved variables.

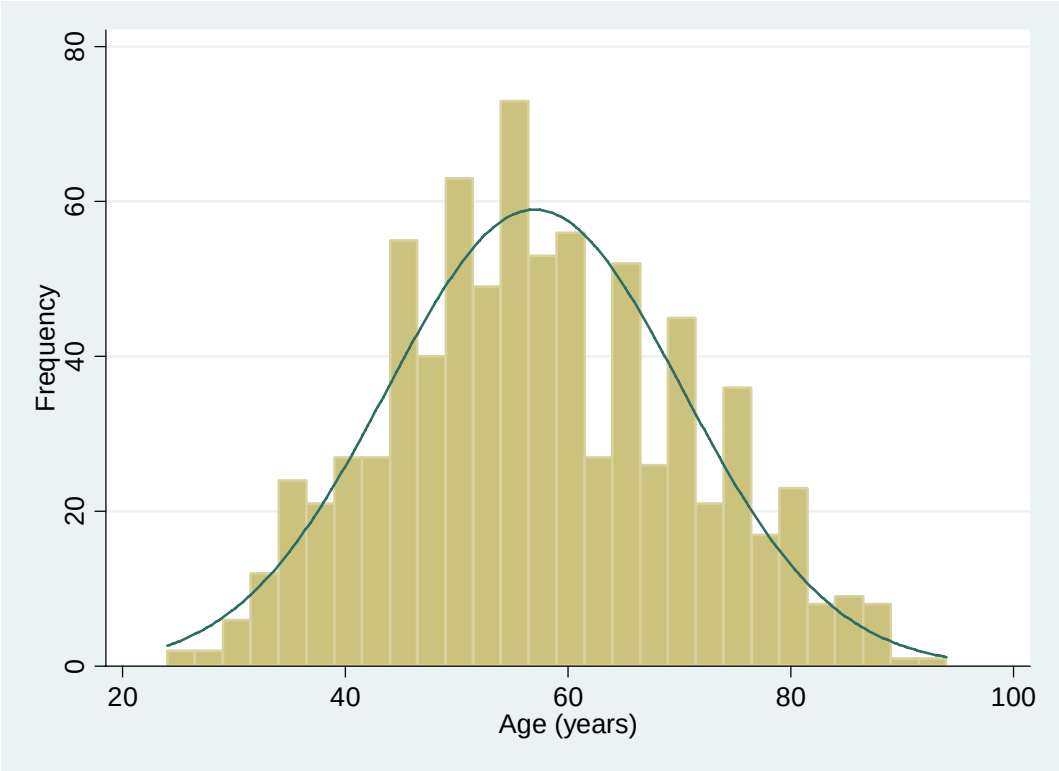
Collinearity diagnostics were adapted using the approach as described by Weissfield[29]. Condition number ≥5 with corresponding variance proportions of ≥0.3 may be indicative of potential collinearity problems.

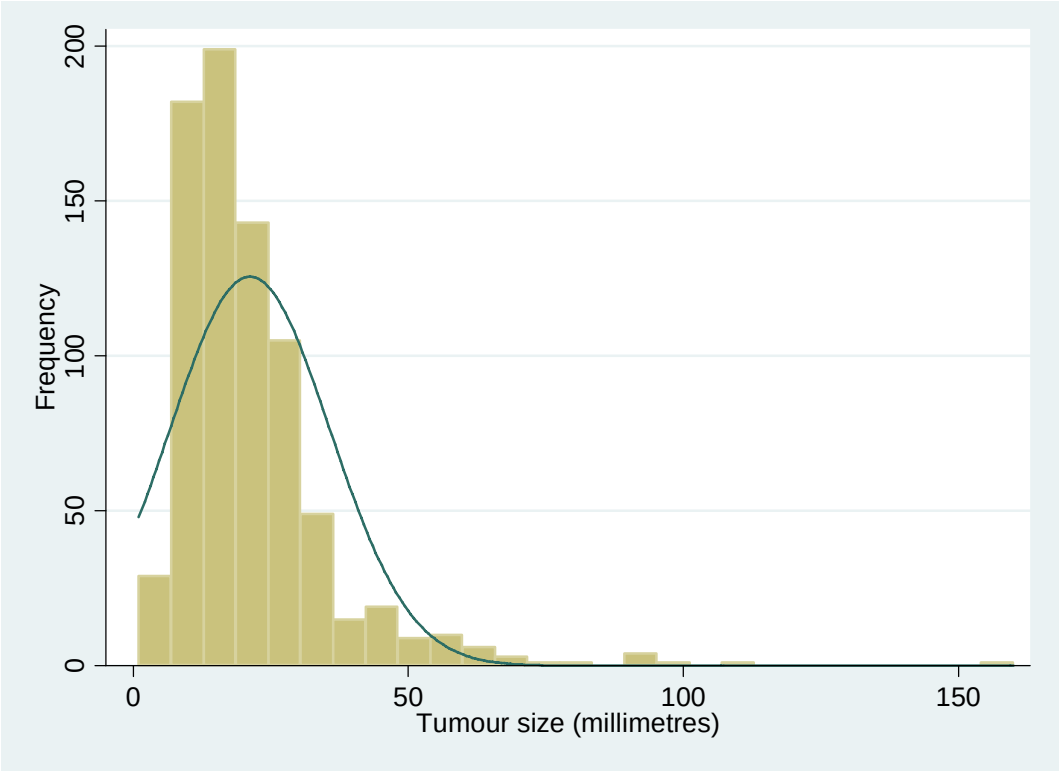
Additional Results

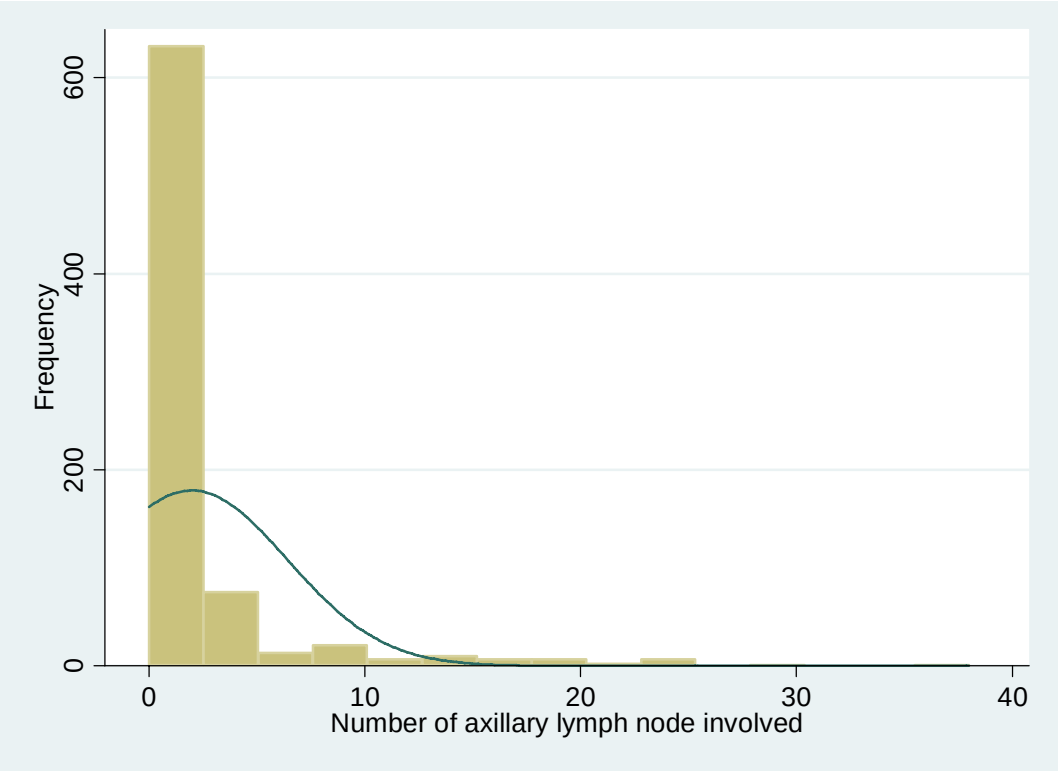
*Baseline characteristics*

Table 1 outlines the baseline characteristics of the patients in this study. Appendix Figure I demonstrates the distribution of the continuous variables.

Figure I - Histograms of the Distributions of Age, Tumour size and Number of Axillary Node Involvement





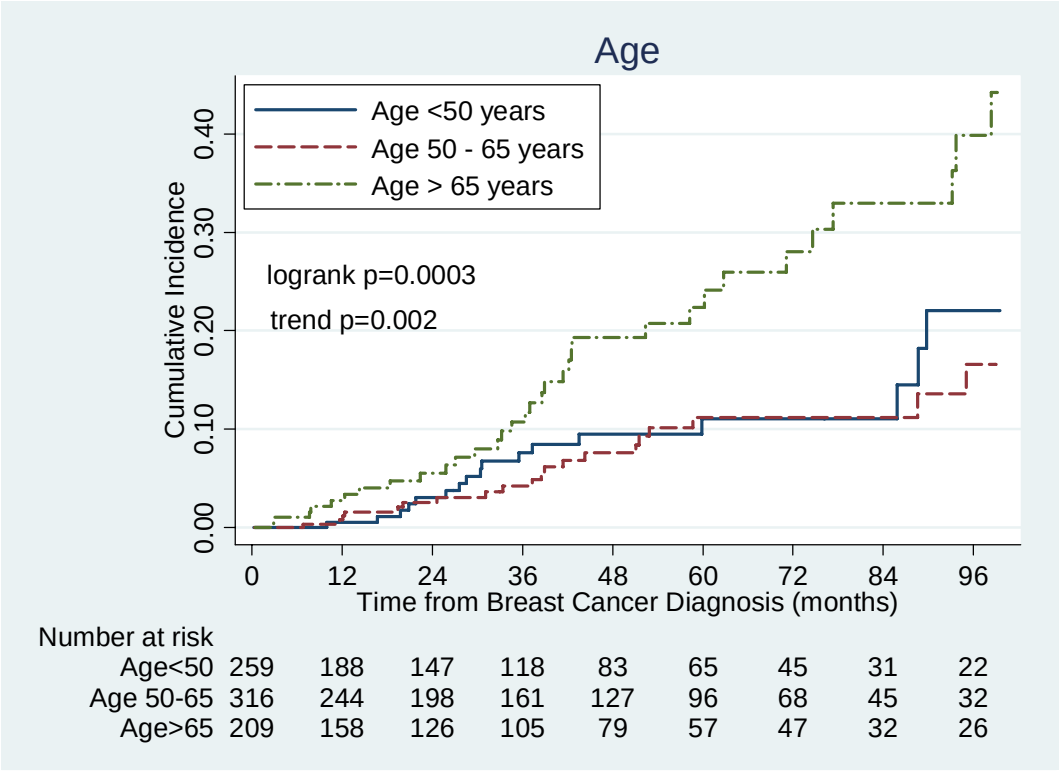

*Univariable Analyses*

When each variable was examined singly, the baseline variables age, performance status, tumour size, axillary lymph node involvement, histologic grade, lymphovascular involvement, endocrine therapy, systemic chemotherapy and radiotherapy were considered as potential predictors of survival ($p \leq 0.20$) (Appendix Table I(a) & Figure I(a)) and were selected for inclusion into the multivariable model. The linearity of the continuously measured covariates was assessed by testing for trend across their categorically ordered groups.

Table I
(a) Univariable Analysis for Overall Survival of the baseline variables

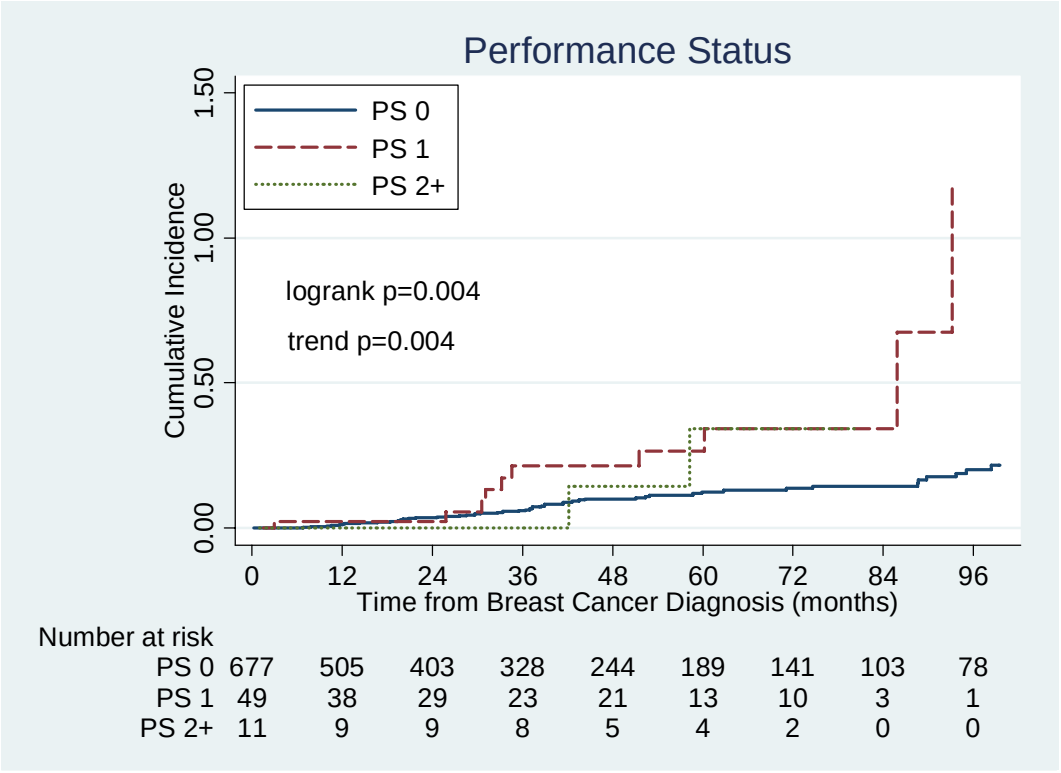
| Characteristics | Hazard Ratio | 95% Confidence Interval | P |
|---|---|---|---|
| Age | 1.04 | 1.02 to 1.06 | <0.001 |
| Premenopause | 1.00 | - | |
| Peri & Postmenopause | 1.01 | 0.77 to 1.31 | 0.96 |
| Performance Status 0 | 1.00 | - | |
| Performance Status 1 | 2.88 | 1.46 to 5.68 | 0.002 |
| Performance Status 2+ | 2.14 | 0.52 to 8.84 | 0.29 |
| Tumour size<20mm (T1) | 1.00 | - | |
| Tumour size 20-49mm (T2) | 2.65 | 1.60 to 4.36 | <0.001 |
| Tumour size ≥50 mm (T3) | 4.35 | 1.76 to 10.71 | 0.001 |
| No axillary lymph node involvement (N0) | 1.00 | - | |
| Axillary 1-3 lymph node involvement (N1) | 2.42 | 1.38 to 4.27 | 0.002 |
| Axillary ≥ 4 lymph node involvement (N2) | 4.69 | 2.67 to 8.22 | <0.001 |
| Well and Moderate differentiated Histologic Grade | 1.00 | - | |
| Poorly differentiated Histologic Grade | 2.34 | 1.47 to 3.74 | <0.001 |
| Absence of lymphovascular invasion | 1.00 | - | |
| Presence of lymphovascular invasion | 2.20 | 1.39 to 3.50 | 0.001 |
| No endocrine treatment | 1.00 | - | |
| Endocrine Treatment | 0.55 | 0.35 to 0.88 | 0.01 |
| No systemic chemotherapy | 1.00 | - | |
| Systemic chemotherapy | 0.72 | 0.47 to 1.11 | 0.14 |
| No radiotherapy | 1.00 | - | |
| Radiotherapy | 0.67 | 0.42 to 1.07 | 0.09 |

Figure I
(a) Selective curves of Cumulative Incidence of Death




Number at risk

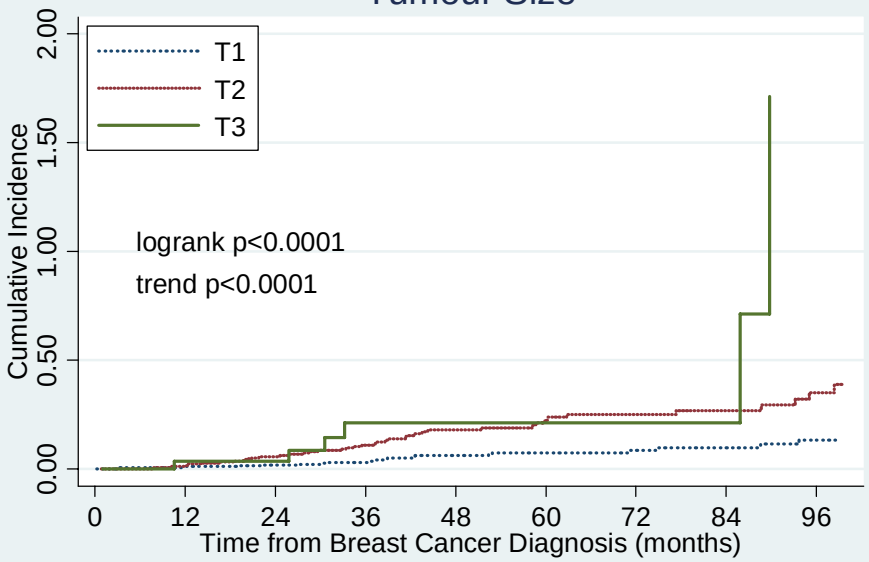
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Age<50 | 259 | 188 | 147 | 118 | 83 | 65 | 45 | 31 | 22 |
| Age 50-65 | 316 | 244 | 198 | 161 | 127 | 96 | 68 | 45 | 32 |
| Age>65 | 209 | 158 | 126 | 105 | 79 | 57 | 47 | 32 | 26 |




Number at risk

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| PS 0 | 677 | 505 | 403 | 328 | 244 | 189 | 141 | 103 | 78 |
| PS 1 | 49 | 38 | 29 | 23 | 21 | 13 | 10 | 3 | 1 |
| PS 2+ | 11 | 9 | 9 | 8 | 5 | 4 | 2 | 0 | 0 |

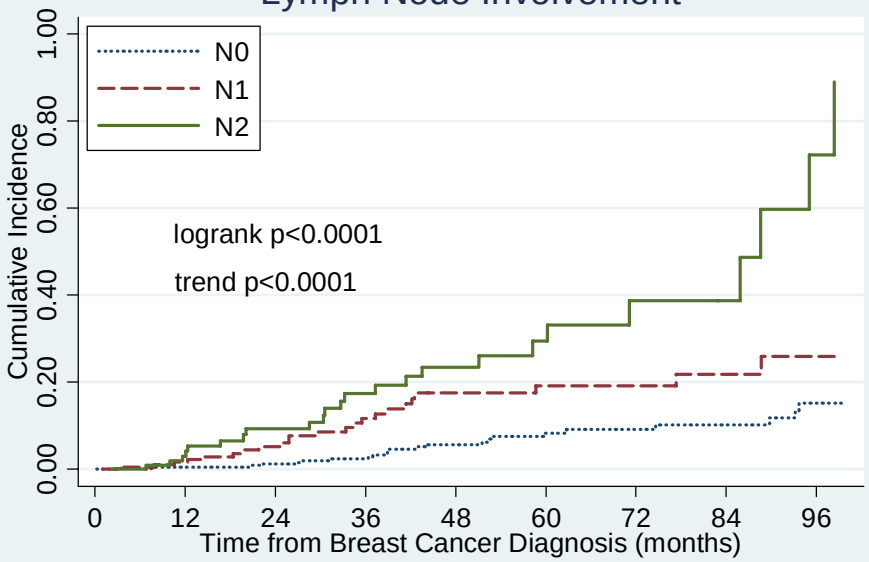
## Tumour Size




Number at risk

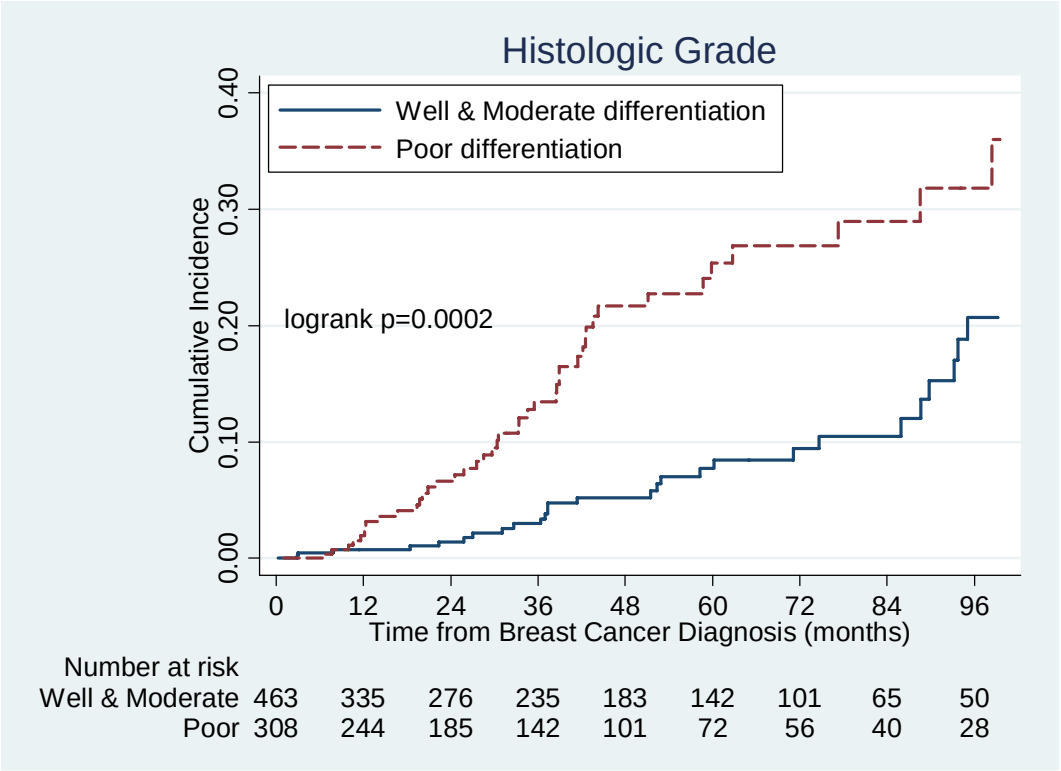
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| T1 | 425 | 311 | 254 | 219 | 168 | 130 | 94 | 60 | 48 |
| T2 | 316 | 249 | 191 | 149 | 107 | 79 | 60 | 45 | 31 |
| T3 | 37 | 25 | 22 | 12 | 11 | 7 | 5 | 2 | 0 |

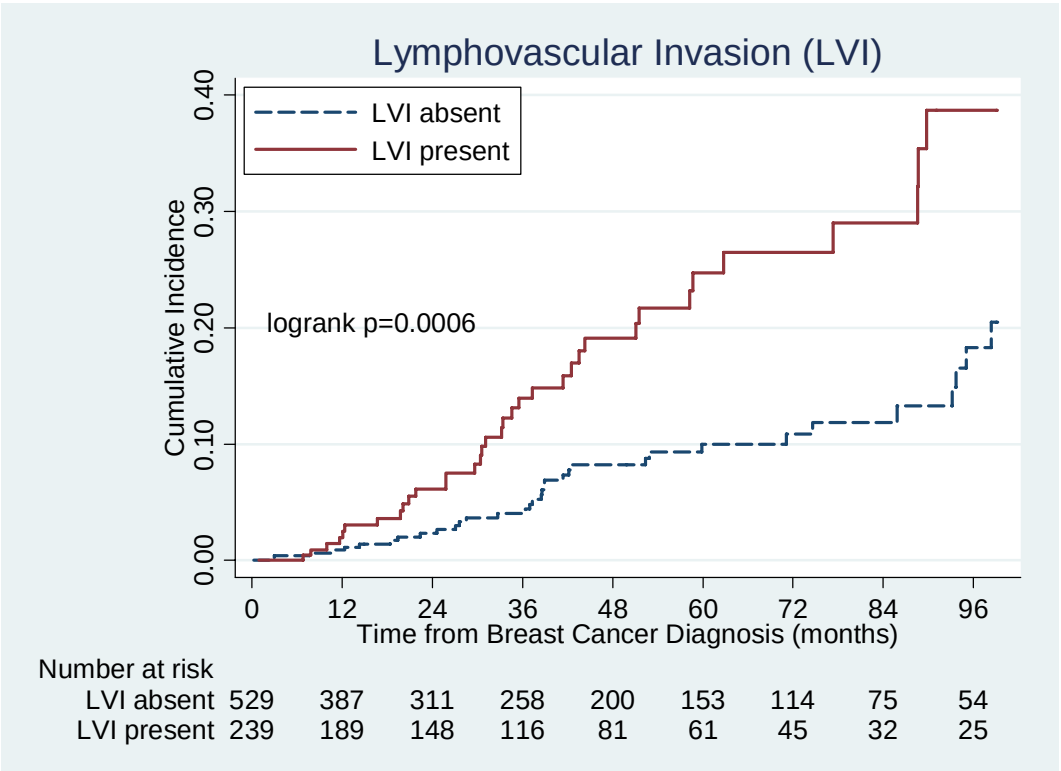
## Lymph Node Involvement

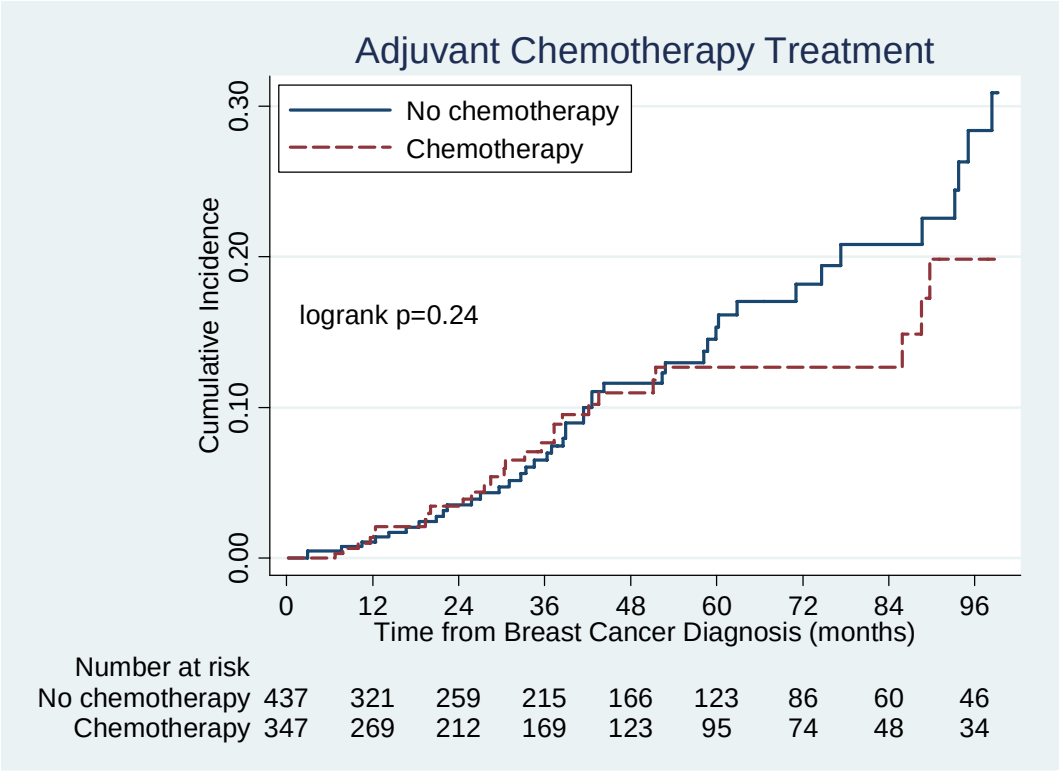



Number at risk

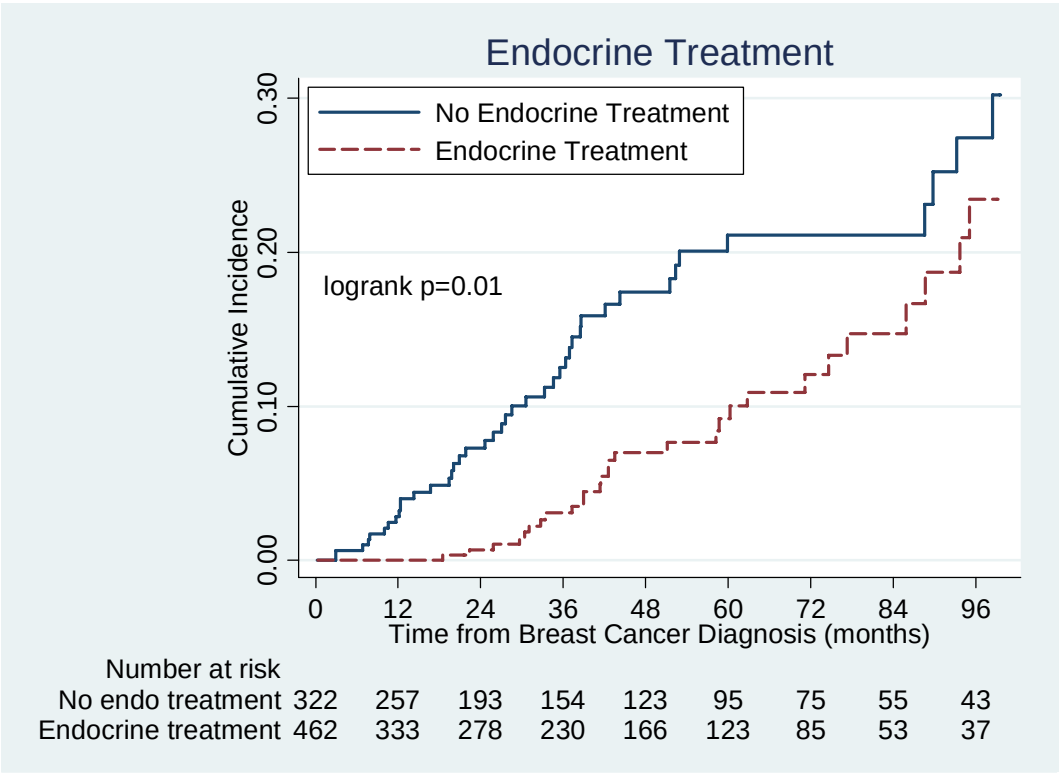
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| N0 | 448 | 336 | 278 | 232 | 180 | 133 | 98 | 68 | 55 |
| N1 | 219 | 163 | 123 | 94 | 68 | 57 | 44 | 28 | 17 |
| N2 | 116 | 90 | 69 | 57 | 40 | 27 | 17 | 11 | 7 |


Histologic Grade
Well & Moderate differentiation
Poor differentiation
logrank p=0.0002
Cumulative Incidence
0.00
0.10
0.20
0.30
0.40
0
12
24
36
48
60
72
84
96
Time from Breast Cancer Diagnosis (months)
Number at risk
Well & Moderate 463 335 276 235 183 142 101 65 50
Poor 308 244 185 142 101 72 56 40 28


Lymphovascular Invasion (LVI)
LVI absent
LVI present
logrank p=0.0006
Cumulative Incidence
0.00
0.10
0.20
0.30
0.40
0
12
24
36
48
60
72
84
96
Time from Breast Cancer Diagnosis (months)
Number at risk
LVI absent 529 387 311 258 200 153 114 75 54
LVI present 239 189 148 116 81 61 45 32 25




| Number at risk | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No chemotherapy | 437 | 321 | 259 | 215 | 166 | 123 | 86 | 60 | 46 |
| Chemotherapy | 347 | 269 | 212 | 169 | 123 | 95 | 74 | 48 | 34 |




| Number at risk | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No endo treatment | 322 | 257 | 193 | 154 | 123 | 95 | 75 | 55 | 43 |
| Endocrine treatment | 462 | 333 | 278 | 230 | 166 | 123 | 85 | 53 | 37 |

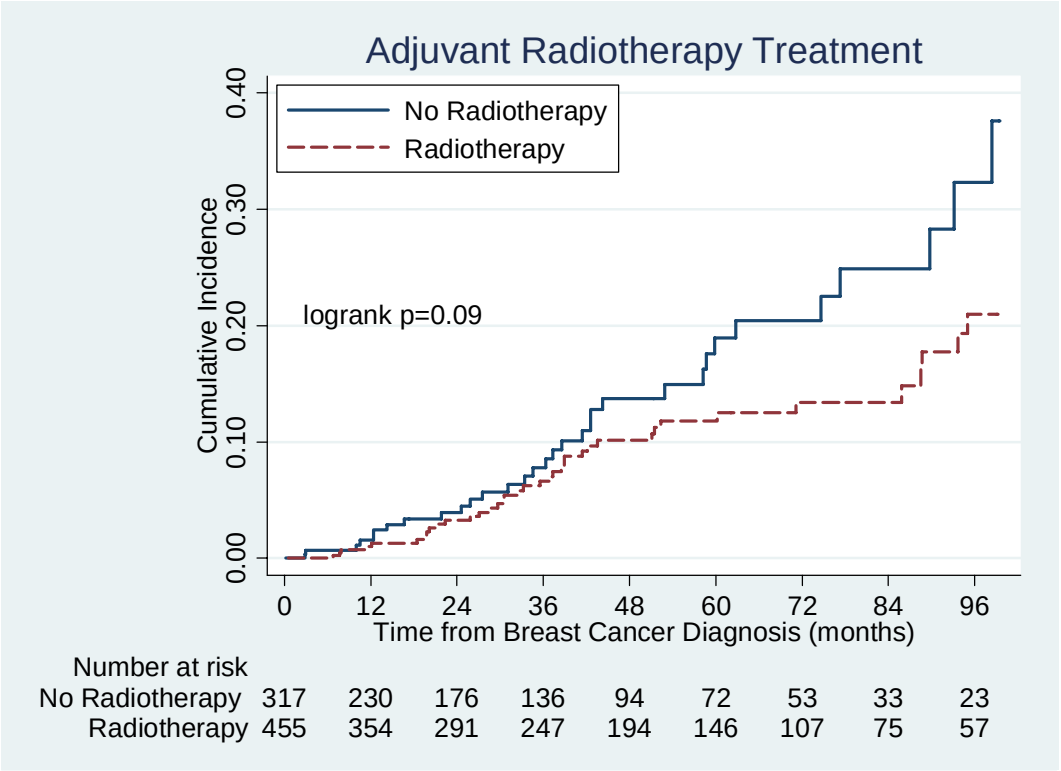
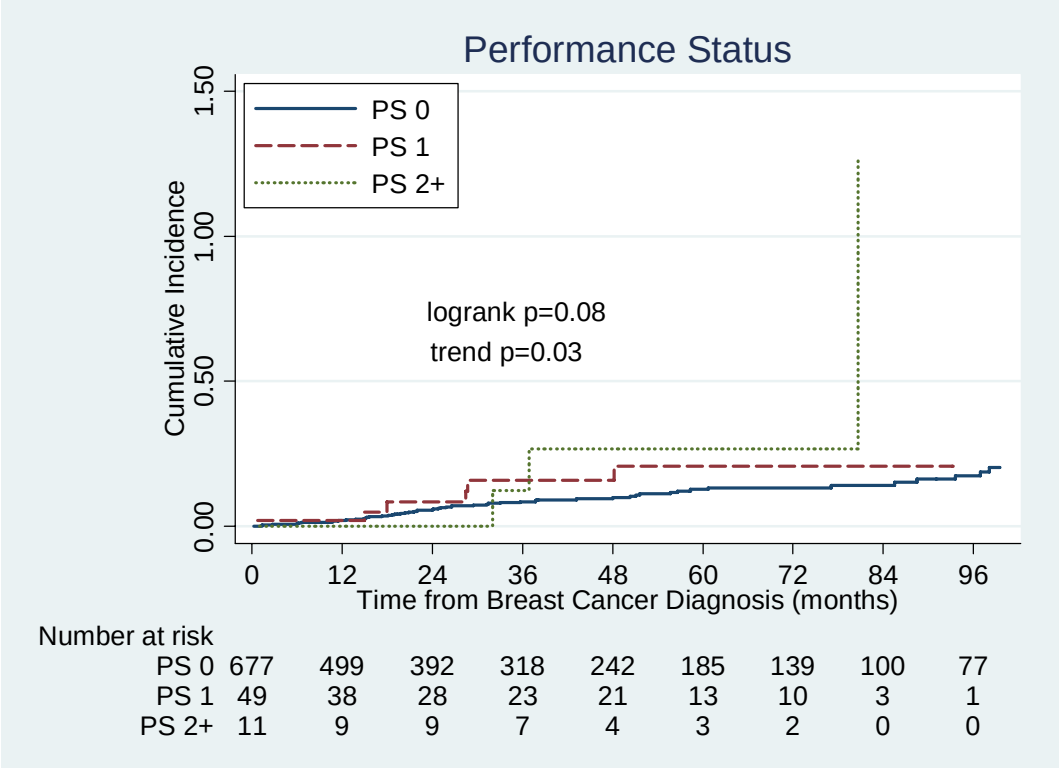
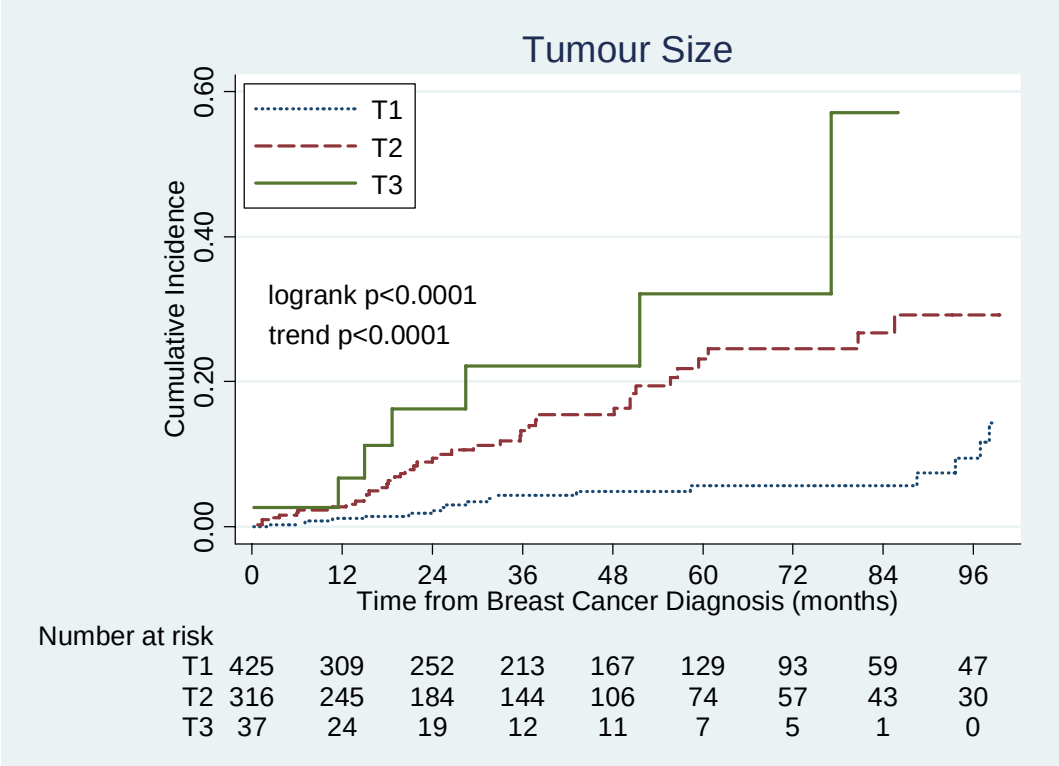

Adjuvant Radiotherapy Treatment
No Radiotherapy
Radiotherapy
logrank p=0.09
Cumulative Incidence
0.00
0.10
0.20
0.30
0.40
0
12
24
36
48
60
72
84
96
Time from Breast Cancer Diagnosis (months)
Number at risk
No Radiotherapy 317 230 176 136 94 72 53 33 23
Radiotherapy 455 354 291 247 194 146 107 75 57

Table I
(b) Univariable Analysis for Recurrence-free Survival of the baseline variables

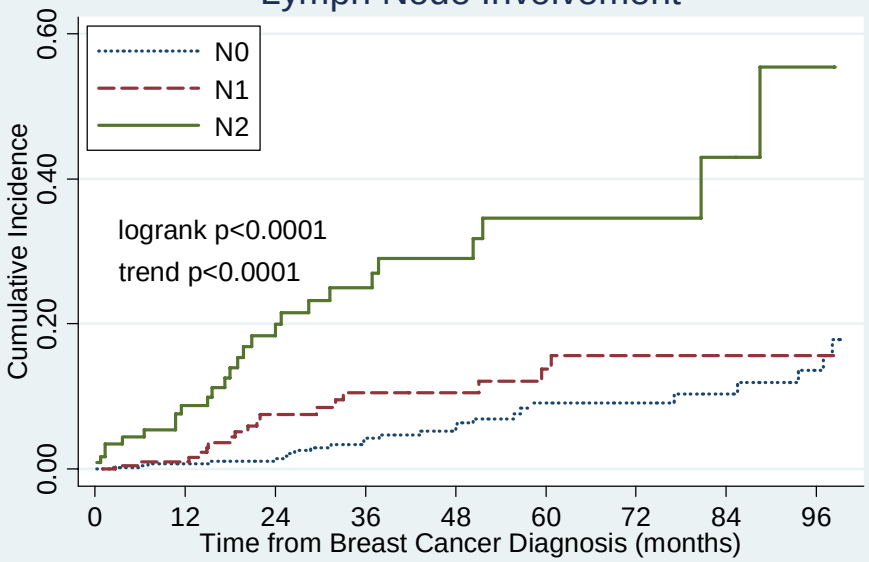
| Characteristics | Hazard Ratio | 95% Confidence Interval | P |
|---|---|---|---|
| Age | 1.01 | 0.99 to 1.02 | 0.57 |
| | | | |
| Premenopause | 1.00 | - | |
| Peri & Postmenopause | 0.88 | 0.68 to 1.14 | 0.32 |
| | | | |
| Performance Status 0 | 1.00 | - | |
| Performance Status 1 | 1.63 | 0.70 to 3.79 | 0.26 |
| Performance Status 2+ | 3.17 | 0.99 to 10.16 | 0.05 |
| | | | |
| Tumour size<20mm (T1) | 1.00 | - | |
| Tumour size 20-49mm (T2) | 2.94 | 1.72 to 5.02 | <0.001 |
| Tumour size ≥50 mm (T3) | 5.44 | 2.29 to 12.91 | <0.001 |
| | | | |
| No axillary lymph node involvement (N0) | 1.00 | - | |
| Axillary 1-3 lymph node involvement (N1) | 1.57 | 0.85 to 2.92 | 0.15 |
| Axillary ≥ 4 lymph node involvement (N2) | 4.67 | 2.69 to 8.10 | <0.001 |
| | | | |
| Well and Moderate differentiated Histologic Grade | 1.00 | - | |
| Poorly differentiated Histologic Grade | 3.46 | 2.06 to 5.80 | <0.001 |
| | | | |
| Absence of lymphovascular invasion | 1.00 | - | |
| Presence of lymphovascular invasion | 3.33 | 2.04 to 5.42 | <0.001 |
| | | | |
| No endocrine treatment | 1.00 | - | |
| Endocrine Treatment | 0.70 | 0.44 to 1.13 | 0.15 |
| | | | |
| No systemic chemotherapy | 1.00 | - | |
| Systemic chemotherapy | 0.97 | 0.76 to 1.23 | 0.80 |
| | | | |
| No radiotherapy | 1.00 | - | |
| Radiotherapy | 0.89 | 0.55 to 1.43 | 0.63 |

Figure I
(b) Selective curves of Cumulative Incidence of Distant Metastasis



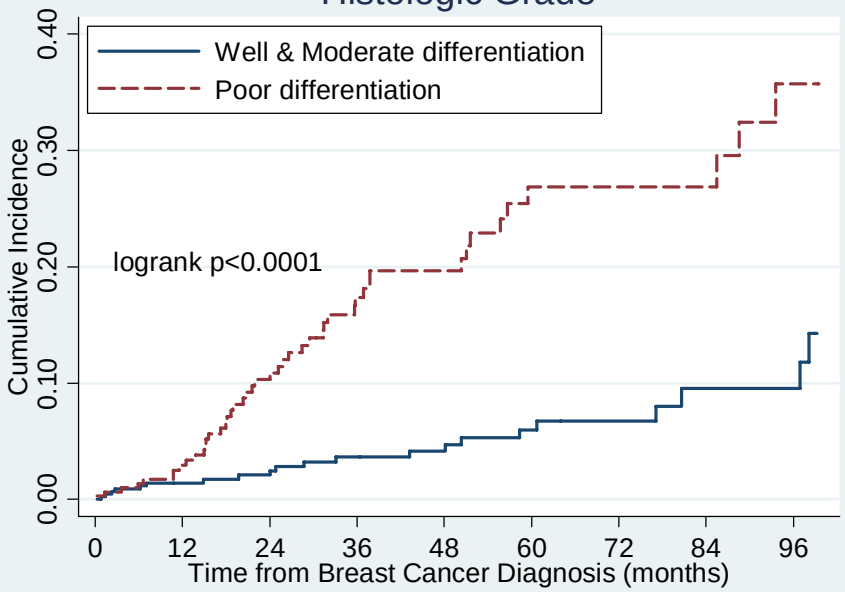
## Lymph Node Involvement




Number at risk

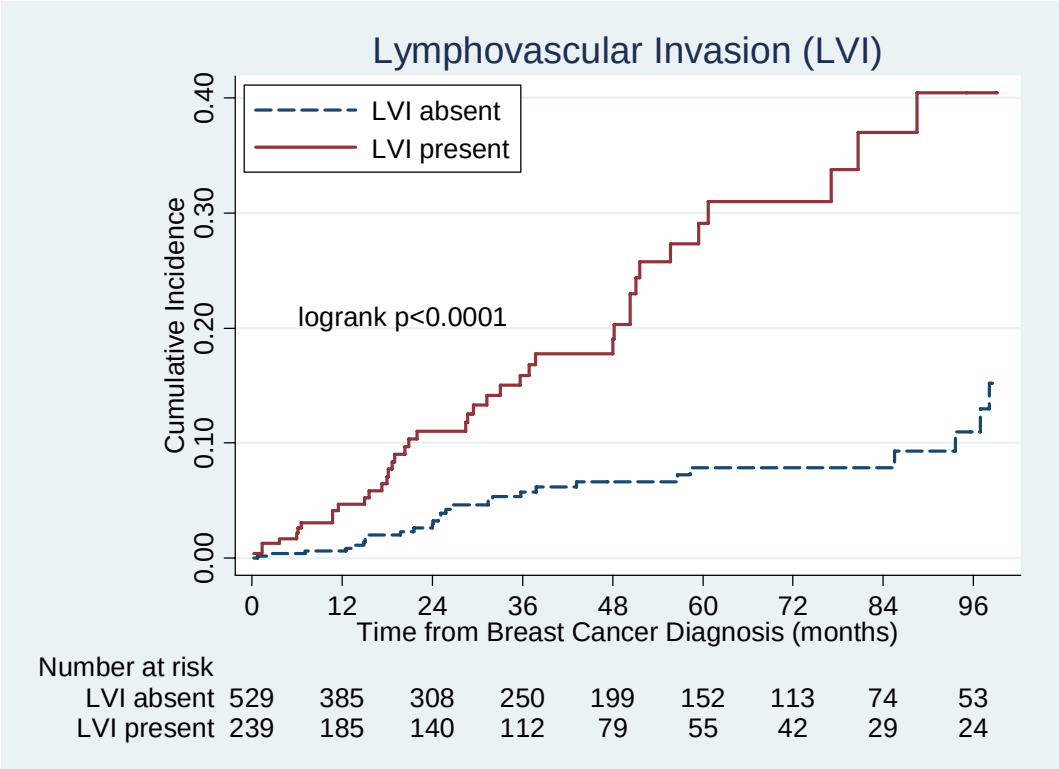
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| N0 | 448 | 333 | 277 | 225 | 179 | 129 | 97 | 66 | 54 |
| N1 | 219 | 163 | 118 | 92 | 68 | 56 | 42 | 27 | 17 |
| N2 | 116 | 86 | 63 | 54 | 38 | 25 | 16 | 10 | 6 |

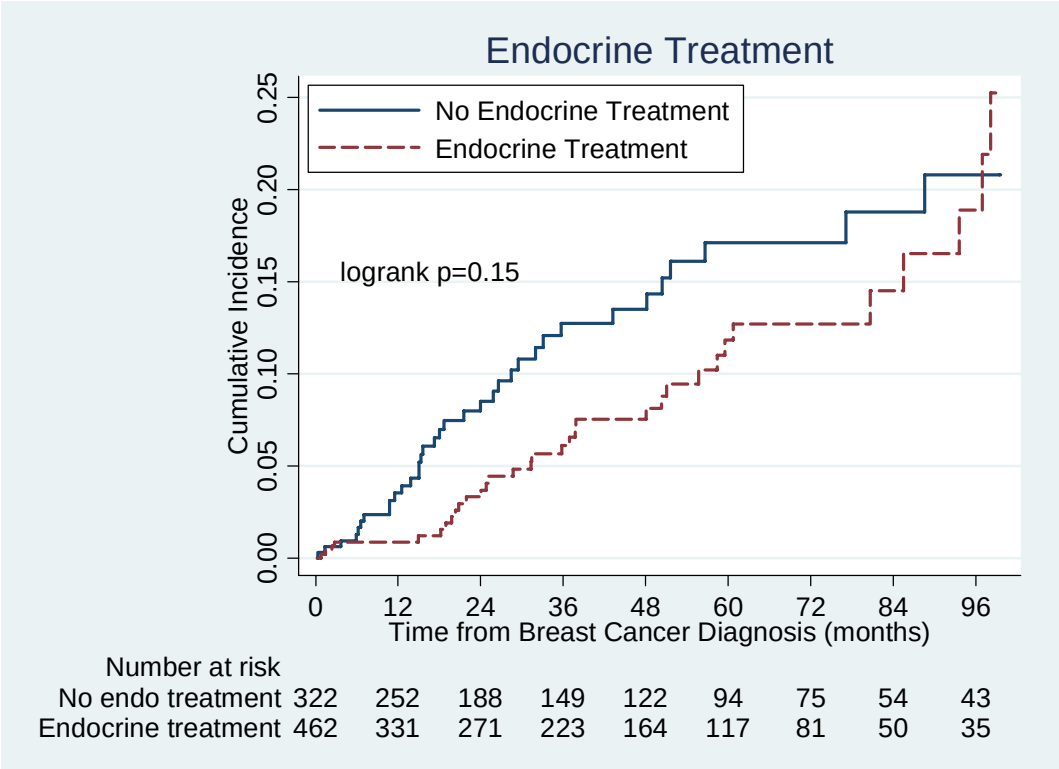
## Histologic Grade




Number at risk

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Well & Moderate | 463 | 331 | 272 | 231 | 182 | 140 | 98 | 61 | 48 |
| Poor | 308 | 241 | 177 | 134 | 99 | 68 | 55 | 40 | 28 |




For the recurrence-free survival outcome, baseline variables performance status, tumour size, axillary lymph node involvement, histologic grade, lymphovascular involvement and endocrine therapy were considered as potential predictors ($p \leq 0.20$) (Appendix Table I(b) &

Figure I(b)) and were selected for inclusion into the multivariable model. The linearity of the continuously measured covariates was assessed by testing for trend across their categorically ordered groups.

*Multivariable Analyses*

The multivariable analyses examined the prognostic significance of breast cancer classification after accounting for other baseline variables. When breast cancer subclass is treated as a factor in the multivariable models, it is statistical significant for survival ($p<0.001$) and recurrence-free survival ($p=0.03$) (Appendix Table IIa & IIb).

Table II
(a) Multivariable Analysis for Overall Survival with baseline parameters in continuous scale

| Characteristics | Hazard Ratio | 95% Confidence Interval | p |
|---|---|---|---|
| Luminal A* | 1.00 | - | - |
| Luminal B* | 0.88 | 0.27 to 2.88 | 0.832 |
| Basal* | 2.86 | 1.63 to 4.99 | <0.001 |
| HER-2* | 4.04 | 1.90 to 8.58 | <0.001 |
| Age (in decades) | 1.32 | 1.08 to 1.61 | 0.008 |
| Tumour Size (mm) | 1.01 | 1.00 to 1.03 | 0.01 |
| Presence of lymphovascular invasion | 1.94 | 1.18 to 3.20 | 0.009 |
| Number of Axillary lymph node involvement | 1.07 | 1.03 to 1.11 | <0.001 |
| Systemic chemotherapy | 0.43 | 0.22 to 0.84 | 0.01 |

* When treated as a factor, breast cancer subgroups remain significant (Wald chunk test: $\chi^2_{(3)}=20.21$, $p=0.0002$) in the multivariable model

(b) Multivariable Analysis for Recurrence-free Survival with baseline parameters in continuous scale

| Characteristics | Hazard Ratio | 95% Confidence Interval | p |
|---|---|---|---|
| Luminal A* | 1.00 | - | - |
| Luminal B* | 0.67 | 0.20 to 2.24 | 0.51 |
| Basal* | 1.31 | 0.67 to 2.58 | 0.43 |
| HER-2* | 2.95 | 1.40 to 6.22 | 0.005 |
| Tumour Size (mm) | 1.01 | 1.00 to 1.03 | 0.03 |
| Presence of lymphovascular invasion | 2.20 | 1.28 to 3.76 | 0.004 |
| Number of Axillary lymph node involvement | 1.07 | 1.04 to 1.10 | <0.001 |
| Poorly Differentiated Histologic Grade | 2.47 | 1.32 to 4.63 | 0.005 |
| Systemic chemotherapy | 0.44 | 0.25 to 0.77 | 0.004 |

* When treated as a factor, breast cancer subgroups remain significant (Wald chunk test: $\chi^2_{(3)}$=8.83, p=0.03) in the multivariable model

Appendix Table II contains the "best" multivariable models developed for survival and recurrence-free survival. Table 3 & 5 (main manuscript) contain refitted multivariable models with categorisation of the tumour size and axillary nodal involvement based on TNM classification[16].

*Proportional Hazards Test*

There was no evidence of proportional hazard violations based on the global test for survival (p=0.30) and recurrence-free survival (p=0.37) models (Appendix Table III). When each variable is examined singly, basal subgroup appears to have borderline proportional hazards violation for survival (p=0.04) and recurrence-free survival (p=0.06) models. However, the plots (Appendix Figure IIa & b) of Schoenfeld residuals versus time-to-events for the basal subgroup suggest that the distributions are centred about zero. Hence, the lack of convincing evidence of proportional hazards assumption violation suggest that simple Cox models

performed in these analyses are adequate to estimate the hazard ratios for the time-to-event data.

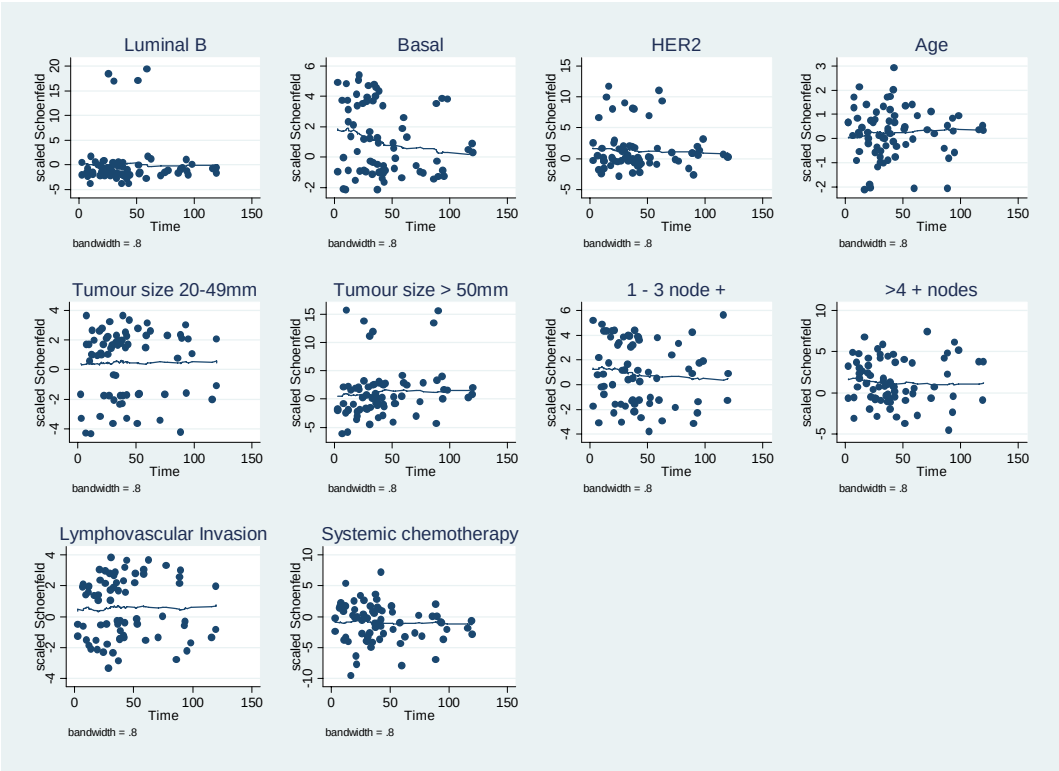
Table III – (a) Proportionality Hazards (PH) Tests for Overall Survival Model

| Characteristics | PH test |
|---|---|
| Luminal B | 0.75 |
| Basal | 0.04 |
| HER-2 | 0.64 |
| Age | 0.88 |
| Tumour size 20-49mm (T2) | 0.76 |
| Tumour size ≥50 mm (T3) | 0.13 |
| Lymphovascular invasion | 0.83 |
| Axillary 1-3 lymph node involvement (N1) | 0.23 |
| Axillary ≥ 4 lymph node involvement (N2) | 0.62 |
| Systemic chemotherapy | 0.28 |

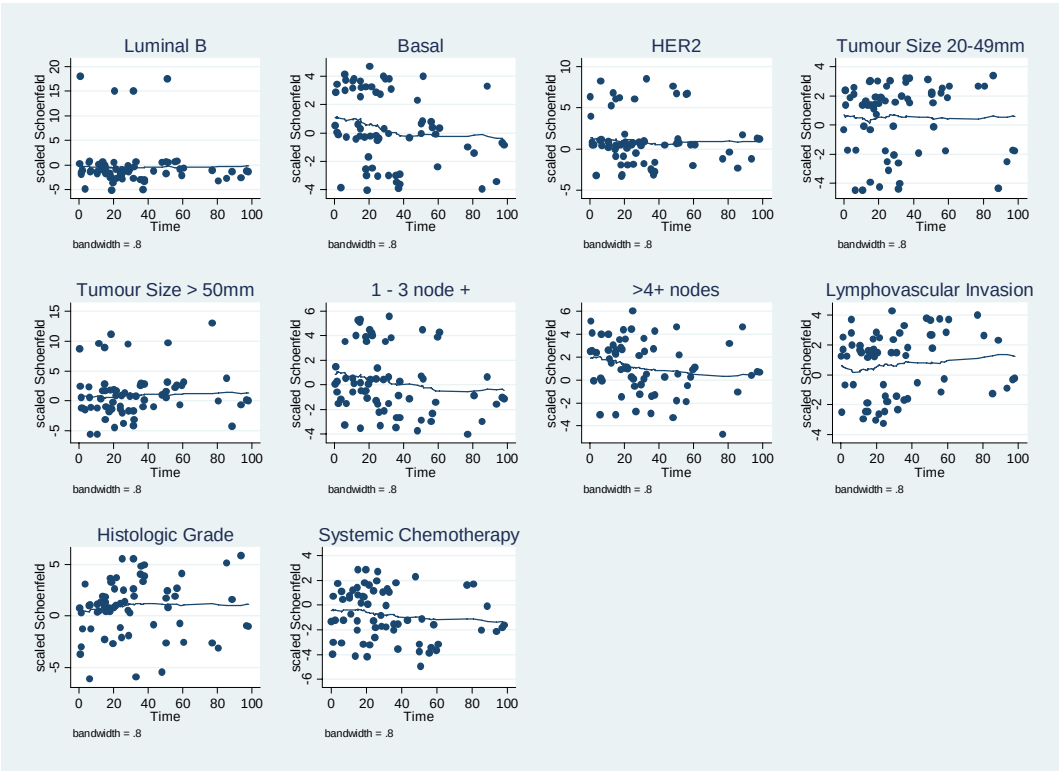
(b) Proportionality Hazard (PH) Tests for Recurrence-free Survival Model

| Characteristics | PH test |
|---|---|
| Luminal B | 0.24 |
| Basal | 0.06 |
| HER-2 | 0.20 |
| Tumour size 20-49mm (T2) | 0.91 |
| Tumour size ≥50 mm (T3) | 0.98 |
| Presence of lymphovascular invasion | 0.53 |
| Axillary 1-3 lymph node involvement (N1) | 0.39 |
| Axillary ≥ 4 lymph node involvement (N2) | 0.05 |
| Histologic Grade | 0.10 |
| Systemic chemotherapy | 0.67 |

Figure II – (a) Scaled Schoenfeld residuals versus survival time – no evidence of non-proportionality




(b) Scaled Schoenfeld residuals versus time to distant metastasis – no evidence of non-proportionality



*Interaction terms*

There was no significant interaction between breast cancer subgroups and other baseline variables in the multivariable models for survival (Wald test $\chi^2_{(19)}$=21.25, p=0.32) or recurrence-free survival (Wald test $\chi^2_{(19)}$=28.74, p=0.07).

*Collinearity Diagnostics*

There was no evidence of collinearity problems in the multivariable models. None of the variables in the multivariable models exhibit large point estimate and inflated standard error (Appendix Table II, Tables 3 & 5 main manuscript). Furthermore, condition indices for these models were less than 5 (Appendix Table IV).

Table IV - Collinearity Diagnostics

(a) Overall Survival Model

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Eigenvalues | 0.273 | 0.409 | 0.572 | 0.695 | 0.864 | 1.044 | 1.199 | 1.373 | 1.625 | 1.947 |
| Condition Number | 2.672 | 2.181 | 1.846 | 1.674 | 1.501 | 1.365 | 1.274 | 1.191 | 1.094 | 1.000 |
| Variance Decomposition Proportion | | | | | | | | | | |
| Luminal B | 0.012 | 0.064 | 0.080 | 0.196 | 0.015 | 0.387 | 0.240 | 0 | 0.005 | 0 |
| Basal | 0.117 | 0.070 | 0.240 | 0.161 | 0 | 0.014 | 0.336 | 0.026 | 0.034 | 0.002 |
| HER2 | 0.211 | 0.061 | 0.216 | 0.113 | 0.020 | 0.246 | 0.056 | 0.002 | 0.037 | 0.039 |
| Tumour size 20-49mm | 0.068 | 0.266 | 0.297 | 0.036 | 0.020 | 0.014 | 0.010 | 0.274 | 0.012 | 0.003 |
| Tumour size ≥50 mm | 0.007 | 0.315 | 0.264 | 0.117 | 0.080 | 0.003 | 0.017 | 0.141 | 0.002 | 0.053 |
| Axillary 1-3 lymph node involvement | 0.329 | 0.188 | 0.099 | 0.070 | 0.123 | 0.008 | 0.009 | 0.031 | 0.141 | 0.002 |
| Axillary ≥ 4 lymph node involvement | 0.485 | 0.296 | 0.043 | 0.004 | 0.001 | 0.006 | 0.008 | 0.001 | 0.137 | 0.020 |
| lymphovascular invasion | 0.029 | 0.030 | 0.092 | 0.192 | 0.524 | 0.005 | 0.001 | 0.067 | 0.010 | 0.050 |
| Systemic chemotherapy | 0.596 | 0.210 | 0.002 | 0.045 | 0.023 | 0.010 | 0.008 | 0.010 | 0.003 | 0.093 |
| Age | 0.394 | 0.131 | 0.013 | 0.172 | 0.099 | 0.085 | 0.009 | 0.005 | 0.019 | 0.072 |

Table IV - Collinearity Diagnostics

(b) Recurrence-free survival Model

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Eigenvalues | 0.328 | 0.381 | 0.523 | 0.68 | 0.863 | 1.037 | 1.256 | 1.376 | 1.574 | 1.982 |
| Condition Number | 2.457 | 2.281 | 1.946 | 1.707 | 1.515 | 1.382 | 1.256 | 1.2 | 1.122 | 1 |
| Variance Decomposition Proportion | | | | | | | | | | |
| Luminal B | 0.073 | 0.143 | 0 | 0.052 | 0.22 | 0.287 | 0.22 | 0.002 | 0 | 0.002 |
| Basal | 0.305 | 0.361 | 0.019 | 0.001 | 0.005 | 0.003 | 0.147 | 0.123 | 0.022 | 0.014 |
| HER2 | 0.344 | 0.215 | 0.016 | 0.005 | 0.037 | 0.149 | 0.003 | 0.215 | 0.010 | 0.005 |
| Tumour size 20-49mm | 0.177 | 0.153 | 0.347 | 0.049 | 0.007 | 0.035 | 0.055 | 0.015 | 0.162 | 0 |
| Tumour size ≥50 mm | 0.075 | 0.186 | 0.508 | 0.008 | 0 | 0.016 | 0.048 | 0.028 | 0.092 | 0.039 |
| lymphovascular invasion | 0.007 | 0.079 | 0.128 | 0.139 | 0.516 | 0.029 | 0.024 | 0.007 | 0.010 | 0.063 |
| Axillary 1-3 lymph node involvement | 0.352 | 0.12 | 0.127 | 0.021 | 0.038 | 0.165 | 0.029 | 0.066 | 0.064 | 0.020 |
| Axillary ≥ 4 lymph node involvement | 0.462 | 0.248 | 0.081 | 0.028 | 0.006 | 0.023 | 0.039 | 0.024 | 0.010 | 0.078 |
| Poorly differentiated Histologic Grade | 0.108 | 0.332 | 0.005 | 0.299 | 0.097 | 0.001 | 0.031 | 0.001 | 0.076 | 0.052 |
| Systemic chemotherapy | 0.181 | 0.003 | 0.137 | 0.497 | 0.024 | 0.038 | 0.002 | 0.028 | 0.006 | 0.084 |

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