Workplace Project Portfolio

for

Master of Biostatistics

University of Sydney

Effect of lymph node yield in sentinel node biopsy for cutaneous melanoma on survival and recurrence

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November 2009

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Scientific Supervisor: A/Prof Andrew Spillane

Principle investigators: Dr Nicholas Lee, Dr Tony Pang

Contents

Table of Contents

Declarations

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

Tony Pang

Date

Supervisor's Declaration

I declare that to my knowledge, this project has been the student, Tony Pang's own work.

Prof Judy Simpson

Date

Context of project and the student's role

This project was not the project I originally arranged for my Workplace Portfolio. After that project with the Cancer Council fell through due to issues with granting me security to their premises after-hours, I found this project through one of the surgeons currently supervising my surgical training, Associate Professor Andrew Spillane. He is an academic melanoma surgeon at the Sydney Melanoma Unit and Royal North Shore Hospital.

The hypothesis of the project arose from the known common practice of some surgeons of retrieving only the lymph nodes which were detectable at operation rather than attempting to retrieve all the nodes detected at lymphoscintigraphy. Hand-held gamma probe, blue dye and surgical intuition were used to find these nodes. It was hypothesized that this surgical (mal)practice may lead to missed detection of nodal involvement and therefore potentially poorer patient outcomes.

At the time of my involvement, the project had already commenced with data collection by Dr Nicholas Lee, a junior surgical trainee. My role was therefore:

- to advise Dr Nicholas Lee regarding important missing data from his dataset and how to obtain them most efficiently;
- Data cleaning and manipulation of data from a datasheet with significant flaws in its original design;
- discussing with Dr Nicholas Lee and A/Prof Spillane regarding the appropriate analysis and implementing it;
- Assisting their interpretation of the results.

Most of my time involved data manipulation to extract information efficiently from the relatively large dataset. All data manipulation and statistical analysis were performed by me.

Due to problems with initial data collection necessitating re-collection of much of the data from a dataset of more than 3000 patients, there were significant delays in commencing data manipulation and analysis. This significantly hampered my ability to consult my statistics supervisor regarding the details of analysis. Despite this, the project was eventually successfully completed albeit slightly late compared to the original timeline.

Preface

This project has been very instructive for my development as a biostatistician. It has allowed me to appreciate that in addition to considering the statistical aspects, there are many other aspects to the biostatistician's work. My reflections on the whole learning experience is documented below.

Communication skills

I played a dual role in this project. Being from a clinical background (surgical trainee in general surgery) I had a unique insight into the aims of the current project and how it may affect future surgical practice. I also had an understanding of what important variables affect survival of melanoma patients and what results are clinically important. However, this advantage was also initially a disadvantage as it made me feel that I should try to handle all aspects of the project myself. This especially occurred in the early part of this project. It also meant that I wrongly assumed that other clinical staff involved in this project had a good grasp of statistical and data management issues.

This led to a major problem when I received the Excel datasheet for analysis. To my surprise the survival and recurrence data collected consisted of a yes/no column of survival and recurrence at 5 years (ie, a binary indicator variable which indicated if death or recurrence occurred within 5 years). At that point I realized the importance of the role of the biostatistician to educate and guide the other team members regarding data and statistical aspects of the project. So after discussion with Dr Lee the main investigator, data collection was recommenced.

From that point on, I started to take a much more active role in communicating with other members of the research team. This included:

- frequent discussion with Dr Lee, who was involved in data collection regarding his progress and how the data can be rapidly retrieved as a large quantity of data required recollection despite a rapidly approaching deadline;
- discussion with the database manager regarding what kind of data is available and how it could be extracted from the database in a form that can be easily manipulated;
- my scientific supervisor regarding the exact question he wanted this study to answer and how we may go about analysing the data to answer this.

As a result, my communication skills were enhanced greatly during the course of this project.

Work patterns/planning

There was a constant great pressure to complete this project as quickly as possible as the unsuccessful commencement of the original project with the Cancer Council meant that this current project only commenced mid-semester. Adding to this narrow timeline was the failure of the initial data collection, and the need to recollect data for more than 3100 patients.

As Dr Lee, A/Prof Spillane and I worked at the same hospital, we were able to arrange frequent communication to ensure no delays in the project. Because of this frequent communication, we were able to deal with any potential delaying issues rapidly. For instance, when it was apparent that follow-up data had to be recollected for all 3113 patients, A/Prof Spillane was able to rapidly arrange a meeting between the database manager, Dr Phil Brown and myself to discuss how the necessary data could be extracted without having to access all 3113 records manually.

Statistical issues, principles and computing

One of the major differences between real life datasets and datasets in books and courses on survival modelling was the presence of missing data. This project greatly increased my understanding in this area, especially in realizing the differences between data MCAR (missing completely at random) and MAR (missing at random). Through additional reading of texts and journal articles, I also learnt of different strategies of dealing with missing data (eg, exclusion, indicator method, single imputation, multiple imputation) and the advantages and disadvantages of each of these. In addition, the experience of multivariate modelling of a disease process with which I am familiar gave me an appreciation of the importance of clinical input in this process rather than relying on a purely mechanical stepwise approach.

This project has also furthered my familiarity with using Stata in applying descriptive analysis (Kaplan-Meier curves), univariate analysis (log rank test), multivariate analysis (Cox proportional hazards models) and model diagnostics (Cox-Snell residuals, Martingale residuals, etc) for survival analysis.

Finally, data manipulation of poorly coded datasets has greatly improved my Excel skills especially in the use of conditional (IF statements) formulae, logical (AND/OR etc) formulae, data lookup formulae (VLOOKUP), and text search and manipulation formulae (MID, FIND commands).

Teamwork

Teaching has become a major part of my role in the team. I was involved in educating the clinical team members and the database manager about some basic survival analysis principles and the data requirements of survival analysis. Also, I noted problems with

coding the data on the datasheet and was able to educate the clinical team member involved in data collection regarding how best to organize and code the data. For instance, when I noticed that some columns in the Excel datasheet consisted of 2 pieces of information, such as "SSM 1.5mm", I was able to educate the team member that it is best to have a column with a code for the tumour type (SSM) and a separate numerical column for depth in mm (1.5).

Ethical considerations

Ethical considerations are also an important part of statistical analysis. This is especially important in experimental studies which involve treatments that are considered non-standard. It is less of an issue in retrospective database analyses like the current study.

For the present study, potential ethical issues were considered. However, according to NHMRC guidelines, as this satisfies the criteria as an audit of an existing database, independent Human Research Ethics Committee (HREC) approval was not required.[1]

Confidentiality and professional responsibility

Confidentiality is an important aspect of any study. As far as possible, data were identified with patient identification numbers (PIN) rather than their names. Addresses and other non-essential personal information were not extracted from the database.

Security of the data depends both on the security of database access and that of the data extracted from the database. Database access was strictly available for investigators of the Sydney Melanoma Unit (SMU) approved research projects. It is available either on site at the SMU or offsite through a password encrypted Citrix platform on the internet. User-specific passwords were issued to the investigators of this project. Data contained within the Excel files were de-identified to ensure patient confidentiality.

Finally, important professional responsibilities such as not accessing unnecessary personal information of subjects, keeping the data secure, and not professing to know or have the ability to do something that I do not know were also adhered to.

Conclusion

Overall, I have learnt a lot from this project. In addition to putting what I have learnt in the Master of Biostatistics into practice, and sharpening my statistical ability, I have learnt much about the non-statistical and professional aspects of being a biostatistician.

Project Report

Abbreviations list

- AL – Acral lentiginous melanoma
- HR – Hazards ratio
- HREC – Human Research Ethics Committee
- LMM – Lentigo maligna melanoma
- LN – lymph node(s)
- LSG – (number of) lymph nodes detected on lymphoscintigraphy
- MAR – (data) Missing at random
- MCAR – (data) Missing completely at random
- MI – Multiple imputation
- NM – nodular melanoma
- PIN – patient identity numbers
- SLN – (number of) sentinel lymph node(s)
- SLNB – Sentinel lymph node biopsy
- SMU – Sydney Melanoma Unit
- SSM – Superficial spreading melanoma

Glossary of medical terms

- Gamma camera – a device used to image a gamma radiation source. It produces an image of the distribution of radionuclide (eg, technetium-99) in the area of interest (eg, lymph node basin);
- Intradermal injection – an injection into the dermal layer of the skin (the layer between the epidermis and subcutaneous tissues);
- Lymph node basin – the collection of lymph nodes where lymph drains into from an anatomical site (eg, the lymph node basin of the arm is the axilla)
- Lymphoscintigraphy – a nuclear medicine imaging test that provides a picture of the lymphatic system using small amounts of radioactive material injected into the skin. This radioactive material travels in the lymph vessels and the radiation released is captured using a gamma camera and recorded to form an image.
- Patent blue dye – a pigment material which is carried in the lymph vessels and dyes them blue temporarily, allowing their identification at surgery.
- Peritumoural injection – an injection of a substance into the vicinity of, but not into, the tumour.
- SPECT (*S*ingle *P*hoton *E*mission *C*omputed *T*omography) – is a special type of nuclear scan which produces a 3-dimensional view of the distribution of radionuclide in the body. It uses a gamma camera to acquire multiple 2-dimensional images at different angles which are then combined by a computer to form a 3 dimensional model.
- Abstract

Introduction: During sentinel lymph node biopsy (SLNB), the yield of sentinel lymph nodes (SLN) is not always equal to the number of nodes identified at lymphoscintigraphy. We hypothesise that this (the "completeness" of SLNB) is related to patient outcome.

Methods: Retrospective case series of 3113 consecutive patients with cutaneous melanoma and SLNB at Sydney Melanoma Unit, a tertiary referral centre. Study period: January 1995-April 2008. Missing data was assumed to be missing at random and multiple imputation (5 imputations) was used to estimate this missing data. Proportional hazards model was used for multivariate analysis. Appropriate model diagnostics were performed. Stratification was used to overcome violations to the proportional hazards assumption.

Results: Median follow-up was 47 (IQR 24 -71) months. Median overall-survival was not reached. Overall 75% survival time was 74 months. 75% disease-free survival time was 44 months. Univariate analysis demonstrated that lymph node yield was associated with improved overall and disease-free survival (P=0.011 and 0.0052 respectively). The multivariate overall survival model was stratified for lymph node location and presence of positive lymph nodes at SLNB. It showed that the lymph node yield did not significantly affect survival (P=0.21, HR=0.88 (95% CI 0.72-1.08)). Male sex, old age, presence of ulceration, high mitotic count, and thicker tumours were associated with poorer overall survival. The multivariate disease-free survival was stratified for age, presence of positive lymph nodes and ulceration. LN yield was again not found to significantly affect disease-free survival (P=0.17, HR=0.90 (95%CI 0.76-1.05). Sex, lymph node location, mitotic count and Breslow thickness were found also to significantly affect disease-free survival.

Conclusion: Lymph node yield at SLNB was not found to significantly affect either overall survival or disease-free survival.

Background

Cutaneous melanoma is a malignant neoplasm of skin melanocytes. In 2002, it was ranked as the 4th most common malignant neoplasm and ninth most common cancer causing death in Australia and New Zealand. Over the period 1991-2003, the incidence of melanoma in Australia showed an upward trend.[2]

The management of melanoma can be divided into the management of:

(1) The primary tumour

(2) The draining lymph node basin

(3) Locoregional recurrence

(4) Distant recurrences/metastases.

The topic of the current study is sentinel lymph node biopsy which is one strategy in the management of occult lymph node disease.

Sentinel lymph node biopsy is a technique used in cancer treatment where the first draining lymph node(s) from the site of the primary tumour, the sentinel node, is sampled to look for metastatic disease. The rationale for this arose from the observation that there is a tendency for melanoma and other cancers to progress in a stepwise orderly manner from primary tumour site, through the local lymph drainage basin then beyond. This stepwise concept of tumour spread was thought also to occur within lymph node basins, in that the first lymph node draining the primary tumour would have a high probability of being involved if lymph node metastasis has occurred.

The very early evidence for this was presented by Morton and colleagues.[3] This was supported by the finding that positive sentinel lymph nodes are associated with early locoregional and distant recurrence.[4]

Despite this, controversy still surrounds sentinel node biopsy and its potential benefits in melanoma patients. While many studies have reported low false negative rates, a recent study has suggested that this may be much higher (between 10-20%).[5] In addition, the third interim analysis of the Multicenter Selective Lymphadenectomy Trial (MSLT1) suggested that, while there was a small melanoma-specific survival improvement at 3 years in the sentinel lymph node group, there was no overall survival benefit.[6]

Could the inconsistencies in the literature be related to quality of surgery, specifically, the lymph node yield at surgery?

Hypothesis

Complete excision of all lymphoscintigraphy-detected sentinel lymph nodes at surgery is associated with improved patient outcomes (overall survival and disease-free survival).

Aims of the study

The three aims of this study are:

- To describe the patients undergoing sentinel lymph node biopsy at the Sydney Melanoma Unit (SMU), particularly, to determine the frequency of incomplete sentinel node biopsies (SNB);
- To assess whether complete SNB is associated with improved disease-free survival; and
- To assess whether complete SNB is associated with improved overall survival.

Methods

This study is a retrospective case series of 3113 consecutive patients who had cutaneous melanoma and a sentinel lymph node biopsy at the Sydney Melanoma Unit (SMU), a tertiary referral centre for melanoma treatment. The study period was from January 1995 to April 2008. These patients were identified from the SMU melanoma patient database, which is a prospectively maintained database of all patients treated at the SMU and includes demographic, clinical, operative, pathological and follow-up data.

Data collected from the database included demographic characteristics (sex, age), sentinel node data (date of sentinel lymph node dissection, area of lymph node basin, number of nodes detected at lymphoscintigraphy, number of nodes actually sampled, number of nodes positive) and primary tumour characteristics (type of growth pattern, Breslow thickness, presence of ulceration, mitotic count).

Formal ethics committee approval was not obtained for this study as it is within the NHMRC definition of an audit.[1]

Brief scientific methods

All patients with biopsy-proven melanoma of thickness greater than 1mm should undergo sentinel lymph node biopsy according to the Clinical Practice Guidelines for the Management of Melanoma.[2]

On the morning of surgery, these patients attended the local nuclear medicine department to undergo lymphoscintigraphy. In this procedure, the primary tumour site was injected intradermally with technetium 99m-labelled colloid and the lymph drainage basins were imaged with a gamma camera. Routine SPECT (Single Photon Emission Computed Tomography) scanning was not utilized. The skin areas over the imaged sentinel nodes ("hot" areas) were marked to assist intraoperative localization.

In the operating theatre just before the commencement of the operation, a 2mL intradermal peritumoral injection of Patent Blue dye (Patent Blue V, Aspen Pharmacare) was performed. A skin incision was placed over the image-detected sentinel nodes and these nodes are carefully dissected out, guided by blue-dyed lymphatic vessels. The sentinel nodes are detected at operation visually (they should be stained blue) and by the use of a hand-held gamma probe. These lymph nodes are excised. Ex-vivo radioactive counts were performed with the gamma probe and any other nodes with a count more than 10% of that of the hottest node or which are dyed blue are considered sentinel nodes even if they were not preoperatively identified by lymphoscintigraphy.

Definitions

Lymph node yield was defined as the number of lymph nodes extracted at surgery divided by the number of lymph nodes detected at lymphoscintigraphy. The number of lymph nodes extracted at surgery was defined as the number of lymph nodes counted on histopathological examination of the operative specimen.

In this study, death was defined as all-cause death. Locoregional recurrence was defined as recurrence at the site of the primary melanoma, in-transit metastases and regional lymph node recurrence. Distant recurrence was defined as distant organ metastases or lymphatic metastases distant from the primary draining lymphatic basin.

For the analysis of overall survival, the event of “failure” was defined as death from any cause. Disease-free survival was defined as survival with no sign of recurrence. For disease-free survival analysis, 3 events were considered “failures”: death from any cause, locoregional recurrence or distant recurrence. Survival times of patients who did not experience any of the relevant failures for each analysis were censored at the time of last follow-up.

Data acquisition and management

The database was located on-site at the Sydney Melanoma Unit, but was accessible remotely through the internet via a secure Citrix server. It is managed by a database manager, Dr Phil Brown. Data were acquired by searching the database for all patients who underwent SNB during the study period. 3127 patients were initially identified.

Microsoft Excel 2007 and Intercooled Stata 9.1 (Statacorp TX USA) were used for data management and cleaning. Intercooled Stata 9.1 was used for all data analysis. The Stata routine, ice, was downloaded and used for multiple imputation of missing data.[7]

The data for the study comprised 2 parts:

- Patient demographic characteristics and details of the operation and pathology findings
- Survival related data (events table) from another table in the database.

Before these data could be analysed, the following procedure had to be followed to extract the appropriate data into an integrated datasheet which could be analysed using Stata.

The basic demographic characteristics and the survival/recurrence data were generated from the database by the database manager using an SQL command to merge a list of patient ID numbers with the relevant tables within the database. However, this meant that there were two Microsoft Excel data tables – an Excel file with 3127 patient and tumour details and a separate Excel file of 25991 separate "events" (ie, treatments, recurrences and deaths) for these 3127 patients. Using a combination of conditional and lookup Excel formulae, I extracted appropriate lists of events (death, recurrence, last follow-up) and copied them into the main dataset.

Two of the original variables were “compound” variables comprised of more than one piece of information for each patient. These were:

- Number of positive lymph nodes, recorded as
    - the number of positive nodes at pathology/number of nodes retrieved, eg, ‘1/2’ or ‘2/3’
- Histological type, recorded as
    - Type and Breslow thickness – eg, ‘SSM 2.3’ for superficial spreading melanoma 2.3mm deep.

These “compound variables” were divided into their separate component variables using conditional FIND and MID text search commands in Excel. Using conditional statements, any inconsistencies were flagged by the text “error” which could be searched for and corrected manually. Any missing data were completed where possible by retrieving the patient’s medical record to extract the relevant data.

Details of Excel commands used in this project are given in Appendix 1.

Data cleaning

Data obtained from the database were checked for errors. The following procedure was undertaken:

- Duplicate records were checked for. The unique key for each patient was the variable “PIN”. This was checked to ensure no duplicates were present in the data.
- Frequency tables were produced for each categorical variable to ensure only possible values were recorded. For example, to ensure that sex was coded either 1 (males) or 0 (females); Clark levels can only be coded 1, 2, 3, 4, 5.

- If a logical relationship exists between variables, this was checked for consistency. For example, there must be fewer positive nodes than the number of lymph nodes extracted at surgery (as the former is a subset of the latter).
- The variable "histological type" was checked to ensure all patients indeed had malignant melanoma.
- For continuous variables, values outside a reasonable or expected range were identified and checked with Dr Lee to confirm accuracy of the data. For example, age outside the range 5-95 years old or Breslow thickness <0 or >15mm. If an error was found in the database and the value could not be confirmed but was considered "impossible", it was replaced by "." (missing value).
- Dates were checked to be in the correct order such that:
    - Date of birth < date of sentinel lymph node biopsy < date of recurrence < date of death or last follow-up.

At the completion of data cleaning and acquisition the following variables were imported into Stata for analysis.

- PIN – patient identification number (unique identifier)
- codemf – patient sex: 1=Male; 0=Female
- dob – Date of birth
- datesnb – Date of sentinel lymph node biopsy
- Age – Age in years calculated as (datesnb-dob)/365.25
- lnarea – lymph node area (1=axilla/upper limb; 2=groin/lower limb; 3=head/neck/axial; 4=multiple)

- lr – left or right side (0=left; 1=right; 2=bilateral)
- LSG – number of lymph nodes detected by lymphoscintigraphy
- SLN – number of lymph nodes removed at surgery
- LNpos – number of sentinel nodes positive for metastatic cancer
- mitosis – number of mitosis per square millimetre
- clarklev – Clark level (1=intraepidermal with intact basement membranes; 2=invasion to papillary dermis; 3=to junction between papillary and reticular dermis; 4=invasion into reticular dermis; 5=invasion into subcutaneous fat)
- ulcer – presence of ulceration (0=absence of ulceration; 1=presence of ulceration)
- Breslow – Breslow thickness (in millimetres)
- type – tumour type (1=superficial spreading; 2=nodular melanoma, 3=acral lentiginous melanoma, 4=lentigo maligna melanoma, 5=desmoplastic)
- lastfup – date of last follow-up or death
- death – censoring variable for last follow-up = status at last follow-up (0=alive; 1=death)
- datedisfree – date of recurrence or last follow-up
- disfree – censoring variable for disease-free survival = status at datedisfree (0=censored; 1=recurrence or death)

Statistical methods

Descriptive statistics were obtained for each variable and tabulated. The number of missing values of each variable was calculated. Median follow-up time was calculated using the actual follow-up periods of each patient, this being the difference in months between the date of sentinel node biopsy and the date of last follow-up. The distribution of each variable was then examined and noted by checking its histogram. Any outliers identified at this point were clarified with the research team.

After data cleaning, imported dates were converted into date format. The time-to-event variables were converted to months from SNB. Overall survival was plotted against months from SNB using a Kaplan-Meier curve with Greenwood 95% confidence interval bands. Descriptive statistics of the overall survival experience were also produced.

Univariate survival analysis of each covariate was performed. Continuous variables were first converted into categorical variables by dividing each into 3 groups of roughly equal size. A log-rank test was then used to compare the survival of groups for each covariate.

Multiple imputation was performed to estimate the missing values. The user-supplied Stata program by Royston (command `ice`) was used.[7] This Stata routine performs imputation by a series of univariable regression models for the conditional distribution of the missing values.[8] The complete dataset was used for the imputation including all patients who had no follow-up data as well as all variables collected for this study. This was so that as much data as possible could be accounted for during the imputation process. Missing values of the time-to-event variable and the censoring variable were included. The time-to-event variables were transformed logarithmically. Count data were also transformed logarithmically as the regression routine of `ice` performs best with normally distributed data. A total of 5 imputed datasets were generated. Missing data were generated by the use of linear regression for continuous data, multilevel logistic regression for categorical data, and ordinal logistic regression for ordered categorical data. The details of the settings for the imputation command are shown in Table 1.

Table 1 – Multiple imputation parameters using "ice".*

Variable	Type	Imputation strategy
Sex (codemf)	Binary categorical	No missing data
Age	Continuous	Linear regression
LN area	Categorical	Multilevel logistic regression
Left/right	Binary categorical	Logistic regression
Log(LSG)	Continuous	Linear regression
Log(SLN)	Continuous	Linear regression
Log(mitosis)	Continuous	Linear regression
Number of LN positive	Count/ordered categorical	Ordinal logistic regression
Clark level	Ordered categorical	Ordinal logistic regression
Presence of ulceration	Binary categorical	Logistic regression
Breslow thickness	Continuous	Linear regression
Type	Categorical	Multilevel logistic regression
Log(months to last follow-up/recurrence)	Continuous	Linear regression
Log(months to last follow-up/death)	Continuous	Linear regression
Overall survival censoring variable	Binary categorical	Logistic regression
Disease-free survival censoring variable	Binary categorical	Logistic regression

*Stata command:

```
.ice codemf Age  lnarea lr  logLSG logSLN logmit LNpos clarklev ulcer Breslow
type  logmtodis logmto fup death disfree using "D:\MBiostat\2009 -
WPP\Clean\data10.dta", cmd(logLSG logSLN logmit:regress LNpos clarklev: ologit,
type: mlogit) m(5)
```

The 5 imputed datasets were then analysed together with the command `micombine`, which computes an average coefficient and standard errors according to the equations suggested by Rubin.[9]

The data were then used to build a Cox proportional hazards model using the "purposeful selection of covariates" method to select the variables.[8] All covariates with

P<0.25 on univariate analysis were selected for the initial multivariate model, along with those covariates considered important in answering our research question. The least significant terms were progressively removed from this model. Variables were retained in the final multivariate model if P<0.05 or they were confounding the effect of other variables on the outcome.

First degree interactions were added to the final reduced model to investigate the presence of effect modification.

The final model was then assessed for:

- presence of appropriate form for continuous variables, by assessing the linearity of the Martingale residuals of a model excluding the covariate of interest;
- validity of the assumption of proportional hazards using Shoenfeld residuals;
- Goodness-of-fit using Cox-Snell residuals.

Note that Stata does not allow the performance of model diagnostics across all five imputed datasets, so the above were only performed on the first imputed dataset. Due to this, it is expected that the residuals calculated would be higher than expected, as the final model is based upon the combined estimate from the models of each imputed dataset rather than on the particular dataset used for residual analysis.

Results

Description of subjects

3127 patients were initially identified in the database. Of these, 14 patients were identified on final pathology to have either a blue naevus or melanoma in-situ. These ineligible patients were excluded, leaving 3113 patients for analysis. The median follow-up time for these subjects was 46.5 months.

Data cleaning resulted in the following adjustments:

- 12 corrected ages
- 1 patient with Clark level "6" (does not exist) was changed to level "5"
- 1 patient where the recurrence date was later than the last follow-up date – the last follow-up date was therefore adjusted to be equal to the date of recurrence.
- 17 tumour types were incorrectly typed in (eg, entered in the datsheet as SM rather than SSM for superficial spreading melanoma).

Patient characteristics are summarised in Table 2. Of note is that there is a predominance of males. Most common lymph node basin is in the axilla and the upper limb nodal basins. The median number of nodes detected at LSG and removed at sentinel node surgery was the same (2 (IQR 1-3)) but the actual range was much greater for the latter (1-9 vs 1-31). The consequent lymph node yield at surgery varied from 0.25 to 15.

The Clark levels and Breslow thicknesses found in the specimen reflect a predominance of intermediate thickness melanoma in our patient population which is the group in which sentinel node surgery is most beneficial.

Table 2 – Summary of patient characteristics and the number of missing data for each variable.

	Number of observations	Number missing (%)	
Demographic characteristics			
Male/female	3113	0 (0%)	Female 1224 Male 1889
Age	3111	2 (0%)	Mean 56.3 (SD 15.8)
Follow-up	2953	160 (5.1%)	Median 46.5 (IQR 24.1-71.2)
Lymph node characteristics			
LN areas	2965	148 (4.8%)	Axilla/upper limb 1365 Groin/lower limb 886 Neck/axial 492 Multiple 202
Left/right	2963	150 (4.8%)	Left 1359 Right 1368 Bilateral 236
Number of nodes on LSG	2961	152 (4.9%)	Median 2 (IQR 1-3) nodes
Number of nodes at surgery	3110	3 (0%)	Median 2 (IQR 1-3) nodes
LN positive for cancer	3110	3 (0%)	0 (0-0) nodes
Characteristics of primary cancer			
Mitoses/mm^2	3069	44 (1.4%)	Median 3 (IQR 1-6)
Clark level	3027	86 (2.8%)	level 1 4 level 2 97 level 3 776 level 4 1912 level 5 238
Presence of ulceration	3070	43 (1.4%)	non-ulcerated 2337 ulcerated 733
Breslow thickness (mm)	3075	38 (1.2%)	Mean 2.43 (SD 1.85)
Tumour type	2540	573 (18.4%)	SSM 1048 NM 1145 AL 56 LMM 48 Desmoplastic 243

Abbreviations: AL = Acral lentiginous melanoma; IQR = interquartile range; LMM = Lentigo maligna melanoma; LN = Lymph node; LSG = Lymphoscintigraphy; NM = nodular melanoma; SSM = Superficial spreading melanoma

Overall survival and disease-free survival

The overall survival experience (with 95% Greenwood confidence bands) of the entire patient cohort is shown in Figure 1. The total number of subjects with follow-up data was 2953 (of 3113, 94.8%). There were 542 deaths with a total time at risk of 154 770 months. The median duration of follow-up was 46.5 months with an interquartile range (IQR) of 24.1 – 71.2 months. The overall median survival time could not be estimated, but the overall 75% survival time was 74 months.

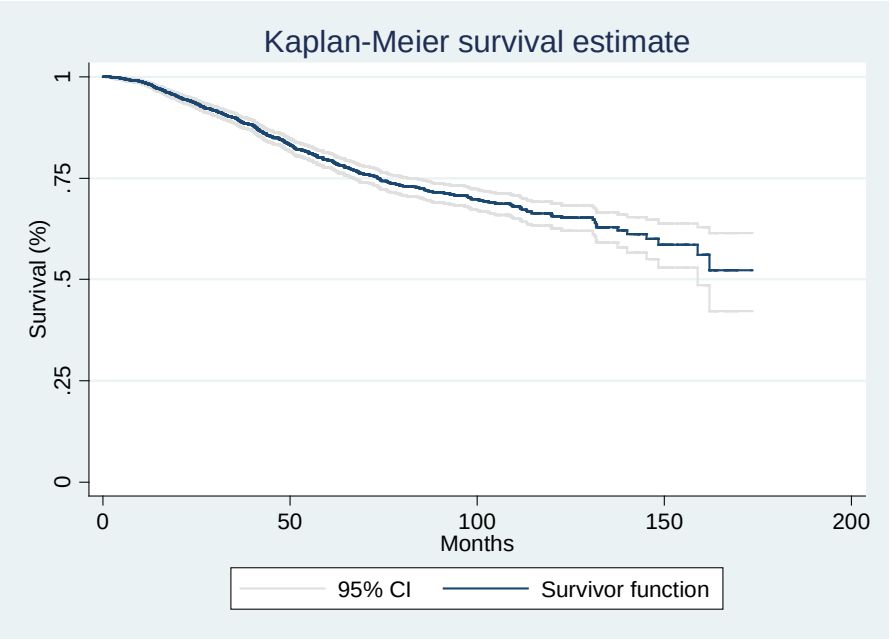


Figure 1 – Kaplan-Meier survival curve for overall survival

Disease-free survival for the entire cohort was also estimated with a Kaplan-Meier survival curve (Figure 2). The 75% disease-free survival time was 44 months.

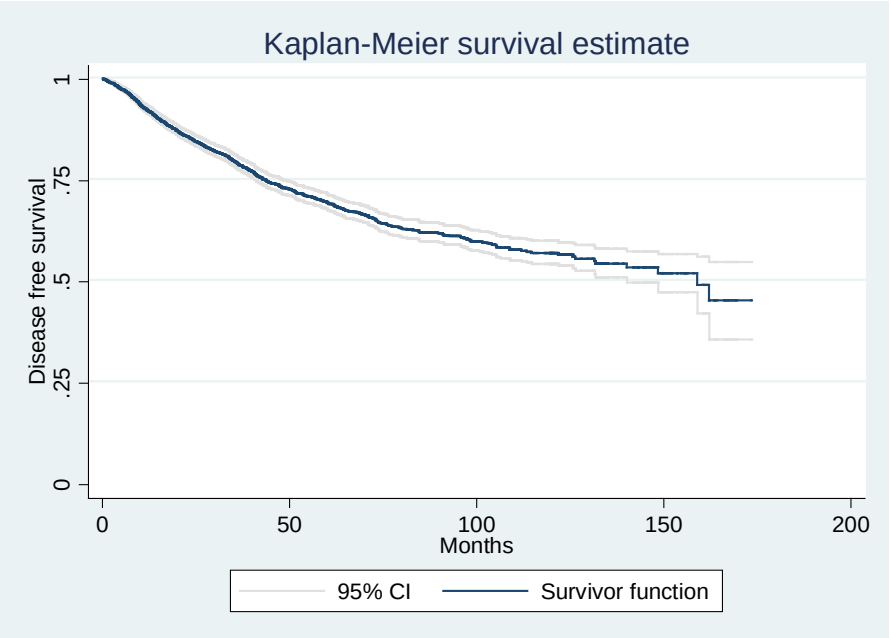


Figure 2 – Kaplan-Meier survival curve for disease-free survival

The results of univariate analysis and the cut-points used for categorisation are shown in the following table (Table 3). Note that the left square bracket means that the value is included and right round bracket means it is not included in range.

In addition to the known factors associated with poor outcome (male sex, old age, presence of ulceration, thickness of tumour, positive lymph nodes), the number of nodes found at initial lymphoscintigraphy and the subsequent yield of lymph nodes at surgery were also found to be predictive factors of survival on univariate analysis. The same factors that significantly affected overall survival also affected disease-free survival significantly.

Table 3 – Summary of univariate analysis for overall survival

Variable	n (with follow-up)	75% survival time (months)	P-value
Sex			<0.0001
Female	1176	104	
Male	1777	64	
Age (years)			<0.0001
[3.4, 50.5)	968	138	
[50.5, 64.9)	988	88	
[64.9- 94.2]	995	49	
LN areas			0.0005
Axilla/UL	1299	87	
Groin/LL	843	73	
Neck/axial	476	54	
Multiple	192	66	
Side of LN basin			0.35
Left	1291	79	
Right	1289	71	
Bilateral	228	77	
Nodes at LSG (n)			0.010
[0, 2)	812	103	
[2, 3)	989	70	
[3, 9]	1005	66	
LN at surgery (n)			0.22
[0, 2)	946	79	
[2, 3)	973	63	
[3, 31]	1031	80	
Ratio of LN at surgery LSG			0.011
<1	602	62	
≥1	2202	79	
Positive nodes (n)			<0.0001
0	2476	97	
≥1	474	36	
Mitosis (n)			<0.0001
[0, 2)	906	112	
[2, 5)	938	88	
[5, 67]	1073	52	

Clark level			<0.0001
1	4	-	
2	91	131	
3	738	103	
4	1811	69	
5	231	45	
Ulceration			<0.0001
Absence	2210	98	
Presence	707	44	
Thickness (mm)			<0.0001
[0, 1.4)	902	145	
[1.4, 2.5)	1030	97	
[2.5, 16)	989	43	
Tumour type			<0.0001
SSM	984	109	
NM	1095	58	
AL	54	42	
LMM	46	72	
Desmoplastic	237	88	

Abbreviations: AL = Acral lentiginous melanoma; LMM = Lentigo maligna melanoma; LN = Lymph node; LSG = Lymphoscintigraphy; NM = nodular melanoma; SSM = Superficial spreading melanoma

Table 4 – Summary of univariate analysis for disease-free survival

Variable	n	75% disease free period (months)	Median disease free period (months)	P-value
Sex				0.0001
Female	1176	54	162	
Male	1778	39	140	
Age (years)				<0.0001
[3.4, 50.5)	968	77	-	
[50.5, 64.9)	988	52	-	
[64.9- 94.2]	996	30	73	
LN areas				<0.0001
Axilla/UL	1300	60	-	
Groin/LL	843	41	159	
Neck/axial	476	30	101	
Multiple	192	44	-	
Side of LN basin				0.13
Left	1292	50	-	
Right	1289	41	159	
Bilateral	228	59	-	
Nodes at LSG (n)				0.012
[0, 2)	812	57	-	
[2, 3)	989	43	149	
[3, 9]	1006	41	162	
LN at surgery (n)				0.91
[0, 2)	946	43	159	
[2, 3)	973	47	-	
[3, 31]	1032	42	162	
Ratio of LN at surgery/LSG				0.0052
<1	602	38	149	
≥1	2203	48	162	
Positive nodes (n)				<0.0001
0	2477	60	162	
≥1	474	13	44	
Mitosis (n)				<0.0001
[0, 2)	906	76	162	
[2, 5)	938	50	159	
[5, 67]	1074	30	102	

Clark level				<0.0001
1	4	-	-	
2	91	52	131	
3	739	65	-	
4	1811	42	159	
5	231	19	71	
Ulceration				<0.0001
Absence	2210	58	167	
Presence	708	20	70.9	
Thickness (mm)				<0.0001
[0, 1.4)	902	126	-	
[1.4, 2.5)	1030	51	162	
[2.5, 16)	990	23	70.9	
Tumour type				<0.0001
SSM	984	73	-	
NM	1096	33	105	
AL	54	13	47	
LMM	46	40	131	
Desmoplastic	237	51	-	

Abbreviations: AL = Acral lentiginous melanoma; LMM = Lentigo maligna melanoma; LN = Lymph node; LSG = Lymphoscintigraphy; NM = nodular melanoma; SSM = Superficial spreading melanoma

Multivariate modelling of overall survival

After performing multiple imputation, a multivariate proportional hazards model was fitted. The initial model for overall survival included the following covariates: sex, age, lymph node basin, nodes found at lymphoscintigraphy, nodes extracted at surgery, whether lymph node yield was 100% or greater (primary outcome of interest), number of positive nodes, number of mitosis, presence of ulceration and tumour thickness. Using the criterion of $P<0.25$ on univariate analysis, all but "side of LN basin" were eligible to be included. However, the following covariates were not included in the initial multivariate model:

- logSLN as this is a figure which is unknown at time of surgery. Also, by knowing LSG and the ratio, logSLN can be defined. Therefore there is duplication of information if it is included.
- Clark levels and tumour types were not included in the initial model despite having a significant effect on outcome on univariate analysis because it is well known clinically that these factors affect patient outcome through the influence of tumour thickness. These two covariates if included will complicate the modelling as each of them is a multilevel categorical variable, with Clark levels being ordered. In addition, both have at least 2 categories with very few patients.

The initial model is shown in Table 5.

Table 5 – Initial model for overall survival

	HR	[95% Conf. Interval]		z	P>|z|
Sex	1.33	1.10	1.60	2.91	0.004
Age	1.03	1.02	1.04	8.55	<0.001
LN location					0.025*
Lower limb vs upper limb	1.29	1.03	1.61	2.22	0.027
Axial vs upper limb	1.42	1.11	1.80	2.85	0.004
Multiple vs upper limb	1.19	0.84	1.70	0.97	0.33
Log(LN detected at LSG)	0.84	0.69	1.03	-1.69	0.091
Ratio of LN at SNB vs LSG (≥1 vs <1)	0.80	0.64	0.99	-2.07	0.038
Number of LN positive	1.89	1.69	2.12	11.14	<0.001
Log(mitotic count)	1.21	1.07	1.37	3.00	0.003
Presence of ulceration	1.43	1.19	1.72	3.78	<0.001
Breslow thickness	1.13	1.09	1.18	6.33	<0.001

Abbreviations: HR = Hazard ratio; LN = Lymph node; LSG = Lymphoscintigraphy; SNB = Sentinel LN biopsy. *overall P-value estimated by Wald test

As can be seen from the above initial multivariate model, one of the LN location categories had the highest P-value. However, using the Wald test, the overall P-value for LN location was 0.025 and a likelihood ratio (LR) test gave LR $\chi^2 = 10.2$ with 3 degrees of freedom (df) and P = 0.017. This suggests that this covariate is an important factor in predicting outcome, so LN location was not deleted from the model.

With the exception of log(LN detected at LSG), all covariates had a significant Wald test (P<0.05). Log(LN detected at LSG) was significant at the 10 percent level and seemed

to be a relatively important clinical variable, so we checked its importance by refitting a model without it. The resulting model is shown in table 6.

Table 6 – Comparison of models with and without log(LN detected at LSG)

	Coeff	P>|z|	Coeff	P>|z|
Sex	0.28	0.004	0.27	0.005
Age	0.03	<0.001	0.03	<0.001
LN location		0.025*		0.065*
Lower limb vs upper limb	0.25	0.027	0.20	0.071
Axial vs upper limb	0.35	0.004	0.31	0.011
Multiple vs upper limb	0.18	0.33	0.10	0.59
Log(LN detected at LSG)	-0.17	0.091	-	-
Ratio of LN at SNB vs LSG (≥1 vs <1)	-0.23	0.038	-0.15	0.14
Number of LN positive	0.64	<0.001	0.62	<0.001
Log(mitotic count)	0.19	0.003	0.19	0.002
Presence of ulceration	0.36	<0.001	0.36	<0.001
Breslow thickness	0.12	<0.001	0.12	<0.001

Abbreviations: Coeff = log(HR); HR = Hazard ratio; LN = Lymph node; LSG = Lymphoscintigraphy; SNB = Sentinel LN biopsy. *overall P-value estimated by Wald test

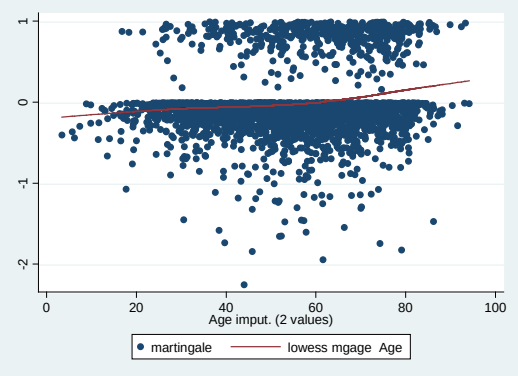
The coefficients for most variables above demonstrate minimal change, but the coefficients for variables describing LN location and the coefficient for LN ratio have changed markedly (>20%). This suggests that log(LN detected at LSG) is an important confounding factor. The LR test (LR $\chi^2 = 3.08$; 1 df) gave P = 0.079. The above therefore suggests that log(LN detected at LSG) is an important covariate which should not be removed from the final model.

At this point, no further variable can be deleted. First-order interactions between LN ratio and other covariates were found not to be significant. Therefore the above formed the final model.

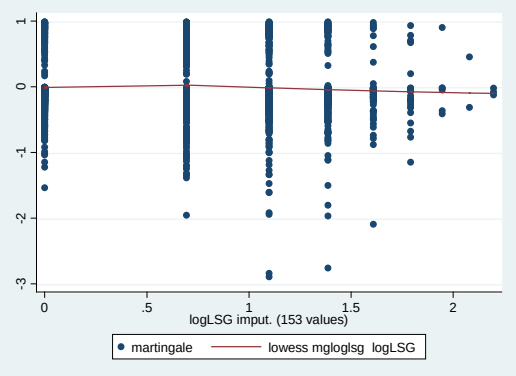
Model diagnostics (for overall survival model)

The functional form of the continuous covariates was checked by plotting the Martingale residuals against the covariate of interest after fitting the Cox model without that covariate.

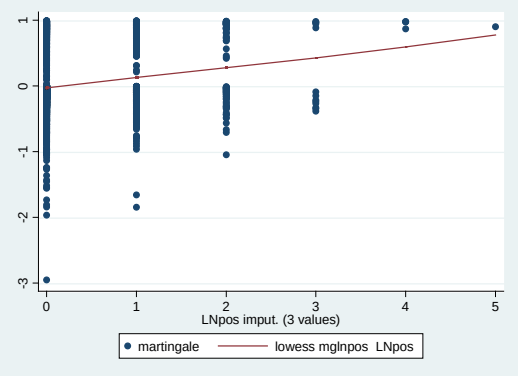
(a)



(b)



(c)



(d)

(e)

Figure 3 – Lowess-smoothed curves for Martingale residuals for models excluding the following variables plotted against the variable itself: (a) Age; (b) log(LN detected at LSG); (c) number of positive LN; (d) log(mitotic count); (e) Breslow thickness.

The plots for the continuous variables, age, log(LN detected at LSG), number of positive LN, log(mitotic count) and Breslow thickness all demonstrated a close to linear Lowess smoothed curve for the Martingale residuals (figure 3). This suggests that the covariates are in an appropriate form and do not require transformation.

Validity of the proportional hazards assumption was tested using scaled Schoenfeld residuals. The results are shown in table 7.

Table 7 – Test of proportional hazards assumption using scaled Schoenfeld residuals.

	rho	chi2	df	Prob>chi2
Sex	0.020	0.22	1	0.64
Age	0.008	0.04	1	0.84
LN location				
Lower limb vs upper limb	0.090	4.73	1	0.03
Axial vs upper limb	-0.039	0.83	1	0.36
Multiple vs upper limb	0.024	0.34	1	0.56
Log(LN detected at LSG)	0.024	0.31	1	0.58
Ratio of LN at SNB vs LSG (≥1 vs <1)	0.027	0.41	1	0.52
Number of LN positive	-0.173	15.6	1	0.0001
Log(mitotic count)	-0.021	0.27	1	0.61
Presence of ulceration	-0.054	1.66	1	0.20
Breslow thickness	-0.011	0.07	1	0.79
Global test		**29.2**	**11**	**0.002**

Abbreviations: HR = Hazard ratio; LN = Lymph node; LSG = Lymphoscintigraphy; SNB = Sentinel LN biopsy.

The above demonstrates that the proportional hazards assumption of the model has been violated. This appears to be mainly due to "number of positive LN" (P=0.0001) but also to the indicator variable for lower limb vs upper limb LN location (P =0.03).

The model was therefore refitted after stratifying for location of lymph nodes and the number of positive LN. Note however that the distribution of the number of positive lymph nodes, shown in figure 4, suggests that while it is in theory a continuous variable, in fact the vast majority of patients had a value of 0 and very few had values greater than 1.

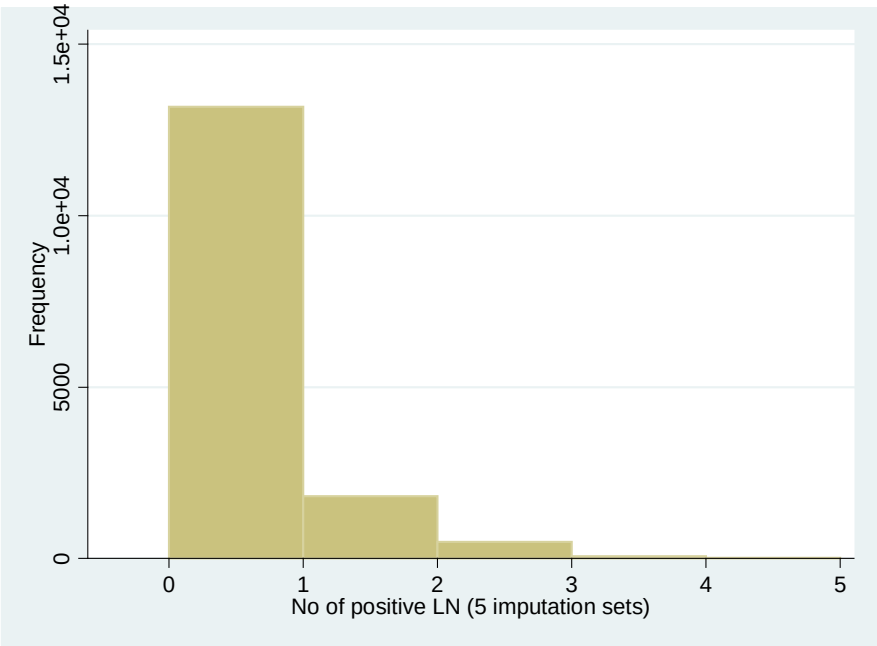


Figure 4 – Histogram demonstrating the distribution of the number of positive lymph nodes found in patients in the study (note that this is a histogram of 5 imputation datasets, therefore, the total number of observations is 15565).

Therefore, I decided to convert this into an indicator variable with 1 indicating the presence of positive lymph nodes and use this binary categorical variable to stratify the data. However, before stratifying the data, the new binary variable was used to refit the model and it was confirmed that this new model still violates the proportional hazards assumption.

After stratifying for lymph node location and the presence of positive lymph nodes at SLNB, the resulting model is shown in table 8.

Table 8 – Stratified final model for overall survival

	HR	[95% Conf. Interval]		z	P>|z|
Sex	1.35	1.11	1.63	3.08	0.002
Age	1.03	1.02	1.03	8.02	<0.001
Ratio of LN at SNB vs LSG (≥1 vs <1)	0.88	0.72	1.08	1.25	0.21
Log(mitotic count)	1.21	1.07	1.37	3.06	0.002
Presence of ulceration	1.42	1.18	1.71	3.76	<0.001
Breslow thickness	1.13	1.08	1.17	6	<0.001

Abbreviations: HR = Hazard ratio; LN = Lymph node; SNB = Sentinel LN biopsy.

The baseline survival curves for the 8 strata are shown in figure 5. As can be seen, LN positive and LN negative curves are quite different from each other. Also, in the LN positive group, different lymph node location groups have different survival curves as well. This is reassuring as it justifies our decision to stratify the data.

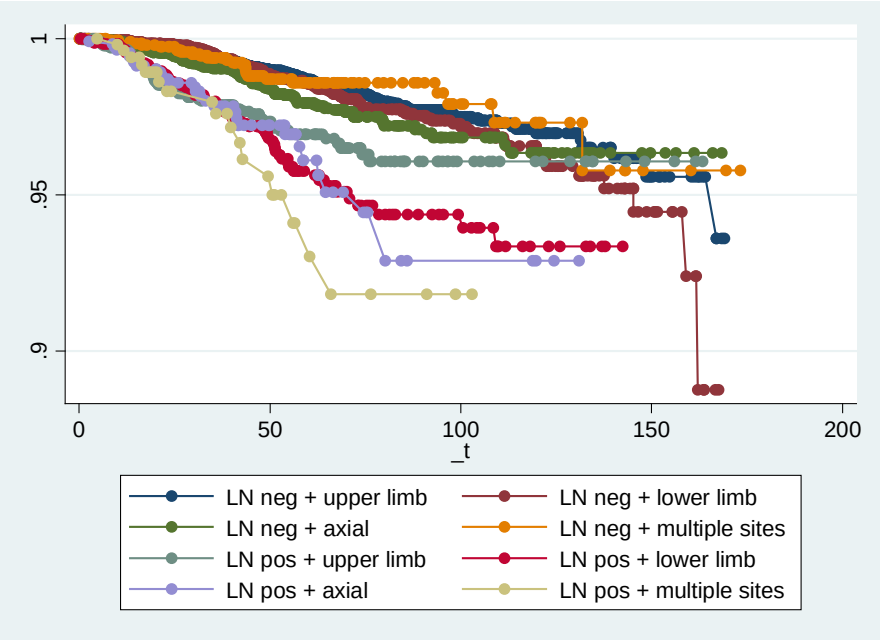


Figure 5 – "Baseline" survival curves for the 8 strata in the final model.

The proportional hazards assumption was tested again using scaled Schoenfeld residuals. This stratified model no longer violates this assumption.

Table 9 – Test of proportional hazards assumption of stratified model using scaled Schoenfeld residuals.

	rho	chi2	df	Prob>chi2
Sex	0.013	0.09	1	0.76
Age	0.030	0.57	1	0.45
Ratio of LN at SNB vs LSG (≥1 vs <1)	-0.012	0.08	1	0.78
Log(mitotic count)	-0.024	0.34	1	0.56
Presence of ulceration	-0.047	1.22	1	0.27
Breslow thickness	-0.015	0.11	1	0.74
Global test		**2.94**	**6**	**0.82**

Abbreviations: HR = Hazard ratio; LN = Lymph node; LSG = Lymphoscintigraphy; SNB = Sentinel LN biopsy.

Overall goodness of fit of the model with Cox-Snell residuals gives the following plot.

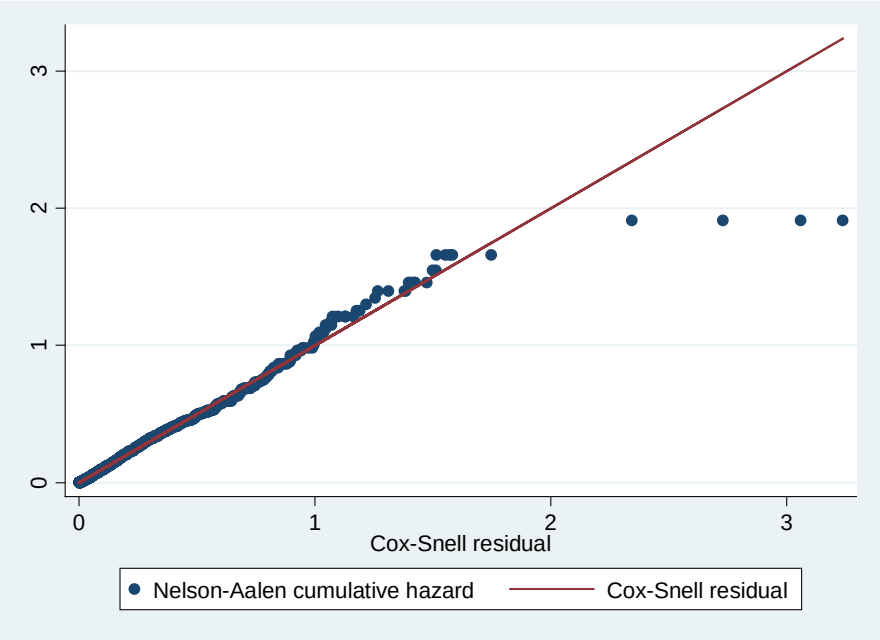


Figure 6 – Cumulative hazard function with Cox-Snell residuals as failure times to test goodness-of-fit of the model.

This indicates that for the vast majority of observations, the goodness of fit of the model is high. Only 4 observations out of 3113 appear not to be fit by the model quite so well.

Multivariate modelling of disease-free survival

The covariates of the initial model (Table 10) were the same as for the initial overall survival model.

Table 10 – Initial model for disease-free survival

	HR	[95% Conf. Interval]		z	P>|z|
Sex	1.20	1.03	1.40	2.33	0.02
Age	1.02	1.02	1.03	9.26	<0.001
LN location					0.0038*
Lower limb vs upper limb	1.29	1.08	1.53	2.83	0.005
Axial vs upper limb	1.33	1.10	1.60	2.93	0.003
Multiple vs upper limb	1.04	0.78	1.39	0.28	0.779
Log(LN detected at LSG)	0.94	0.80	1.09	-0.84	0.402
Ratio of LN at SNB vs LSG (≥1 vs <1)	0.84	0.71	1.00	-1.94	0.053
Number of LN positive	1.79	1.62	1.97	11.5	<0.001
Log(mitotic count)	1.18	1.08	1.29	3.7	<0.001
Presence of ulceration	1.34	1.15	1.55	3.79	<0.001
Breslow thickness	1.12	1.09	1.16	7.06	<0.001

Abbreviations: HR = Hazard ratio; LN = Lymph node; LSG = Lymphoscintigraphy; SNB = Sentinel LN biopsy. *overall P-value estimated by Wald test

The model was reduced by in turn removing the log(LN detected at LSG), as shown in table 11.

Table 11 – Reduced model for disease-free survival

	HR	[95% Conf. Interval]		Z	P>|z|
Sex	1.20	1.03	1.40	2.29	0.022
Age	1.02	1.02	1.03	9.24	<0.001
LN location					0.0053*
Lower limb vs upper limb	1.26	1.07	1.49	2.7	0.007
Axial vs upper limb	1.30	1.08	1.57	2.82	0.005
Multiple vs upper limb	1.01	0.76	1.34	0.07	0.94
Ratio of LN at SNB vs LSG (≥1 vs <1)	0.87	0.74	1.02	-1.75	0.081
Number of LN positive	1.77	1.61	1.95	11.55	<0.001
Log(mitotic count)	1.19	1.08	1.30	3.75	<0.001
Presence of ulceration	1.34	1.15	1.56	3.8	<0.001
Breslow thickness	1.12	1.09	1.16	7.01	<0.001

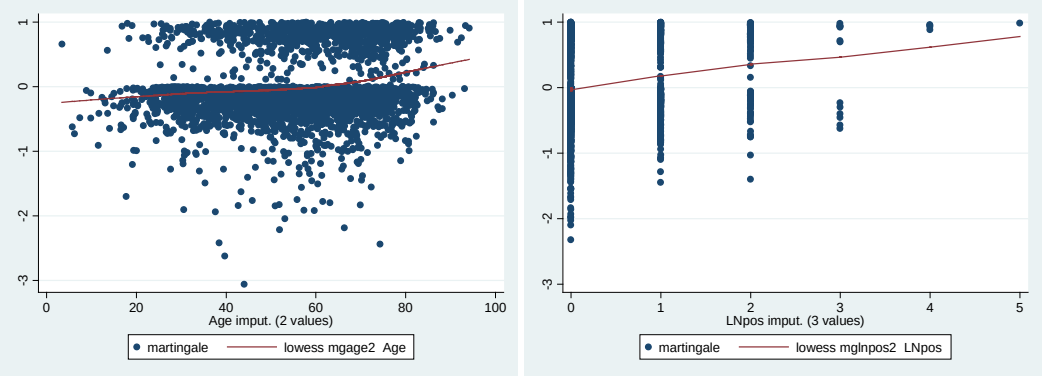
Abbreviations: HR = Hazard ratio; LN = Lymph node; LSG = Lymphoscintigraphy; SNB = Sentinel LN biopsy. *overall P-value estimated by Wald test

In this model, the largest P-value is 0.081 for LN ratio. As this is the variable of interest of the study it was kept in the model. No further reduction in this model is possible. First-order interactions of LN ratio with other covariates were not significant, so this model forms the final model.

Model diagnostics for the disease-free survival model

The functional form of the continuous covariates was checked in the same way as for the overall survival model. The plots for age, number of positive LN, log(mitotic count) and Breslow thickness all demonstrated a close to linear Lowess smoothed curve suggesting that the covariates are in an appropriate form and do not require transformation (figure 7).

(a) (b)



(c) (d)

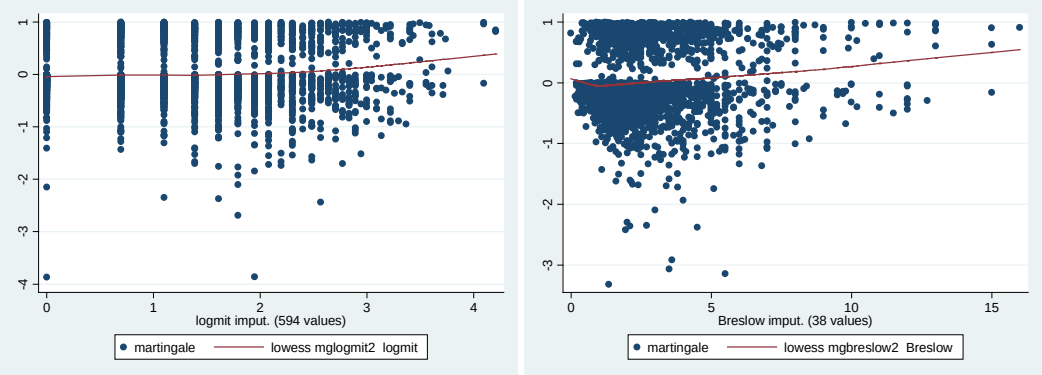


Figure 7 – Lowess-smoothed curves for Martingale residuals for models excluding the following variables plotted against the variable itself: (a) Age; (b) number of positive LN; (c) log(mitotic count); (d) Breslow thickness.

Using Schoenfeld residuals, the proportional hazards assumption was tested (Table 12). Age, presence of ulceration, and number of lymph nodes positive were found to have violated this assumption.

Table 12 – Test of proportional hazards assumption with Schoenfeld residuals.

	rho	chi2	df	Prob>chi2
Sex	0.012	0.1	1	0.720
Age	0.068	4.9	1	0.026
LN location				
Lower limb vs upper limb	0.018	0.3	1	0.594
Axial vs upper limb	-0.060	3.2	1	0.074
Multiple vs upper limb	-0.042	1.6	1	0.21
Ratio of LN at SNB vs LSG (≥1 vs <1)	0.016	0.2	1	0.63
Number of LN positive	-0.149	18.0	1	<0.0001
Log(mitotic count)	-0.030	0.9	1	0.348
Presence of ulceration	-0.104	9.6	1	0.002
Breslow thickness	-0.009	0.1	1	0.797
Global test		49.28	10	<0.0001

Abbreviations: HR = Hazard ratio; LN = Lymph node; LSG = Lymphoscintigraphy; SNB = Sentinel LN biopsy. *overall P-value estimated by Wald test

The model was therefore stratified according to the above variables. The number of lymph node positive was categorised as for the analysis of overall survival. Age was also categorised into the 3 categories used in univariate analysis (table 3). This new model with 12 strata is shown in table 13.

Table 13 – Stratified model for disease-free survival

	HR	[95% Conf. Interval]		Z	P>|z|
Sex	1.22	1.05	1.43	2.53	0.011
LN location					0.0076*
Lower limb vs upper limb	1.24	1.05	1.47	2.53	0.011
Axial vs upper limb	1.32	1.09	1.58	2.9	0.004
Multiple vs upper limb	1.04	0.78	1.38	0.25	0.805
Ratio of LN at SNB vs LSG (≥1 vs <1)	0.90	0.76	1.05	-1.38	0.17
Log(mitotic count)	1.18	1.08	1.30	3.71	<0.001
Breslow thickness	1.12	1.08	1.16	6.83	<0.001

Abbreviations: HR = Hazard ratio; LN = Lymph node; LSG = Lymphoscintigraphy; SNB = Sentinel LN biopsy. *overall P-value estimated by Wald test

Rechecking the proportional hazards assumption gives table 14. This demonstrates that whilst two LN location dummy variables were almost significant for violation of the proportional hazards assumption, neither was. The global test also was non-significant (P=0.24). We also investigated the possibility of refitting the model with LN location stratified, but found that the coefficients and P-values of the remaining variables were minimally changed but the model had 3 times the number of strata of the existing model (ie, 36 strata). Therefore, by the principle of parsimony, we decided not to further stratify the model.

Table 14 – Proportional hazards assumption tested for the final stratified model for disease-free survival

	rho	chi2	df	Prob>chi2
Sex	0.007	0.04	1	0.84
LN location				
Lower limb vs upper limb	0.011	0.12	1	0.73
Axial vs upper limb	-0.062	3.36	1	0.07
Multiple vs upper limb	-0.063	3.56	1	0.06
Ratio of LN at SNB vs LSG (≥1 vs <1)	0.014	0.18	1	0.67
Log(mitotic count)	-0.022	0. 48	1	0.49
Breslow thickness	-0.002	0.00	1	0.96
Global test		**9.19**	**7**	**0.24**

Abbreviations: HR = Hazard ratio; LN = Lymph node; LSG = Lymphoscintigraphy; SNB = Sentinel LN biopsy. *overall P-value estimated by Wald test

Overall goodness of fit of the model with Cox-Snell residuals gives the following plot.

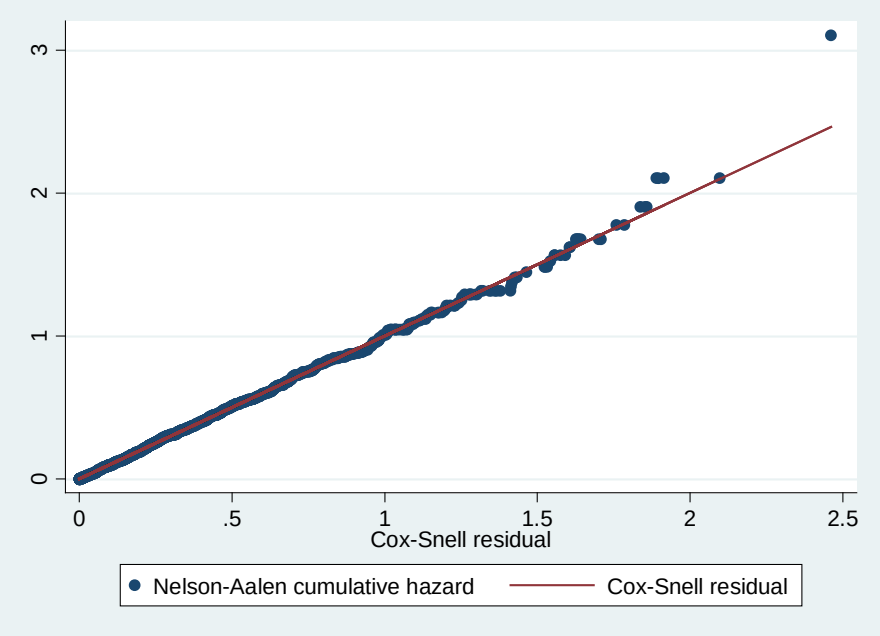


Figure 8 - Cumulative hazard function with Cox-Snell residuals as failure times to test goodness-of-fit of the model for disease-free survival.

Again, like the overall survival model, the goodness-of-fit is high for the vast majority of the observations (except for one out of 3113).

Comparison with complete data only analysis

To confirm that the process of multiple imputation has not produced unexpected results, analysis was repeated with patients with completed data only. Using the same covariates as in the models developed above after multiple imputation, Cox proportional hazards models were estimated using patients with complete data only. The results are summarized below (table 15 for overall survival, table 16 for disease-free survival).

Table 15 – Comparison between models for overall survival using complete data only (complete) and using imputed missing data (MI)

	HR (MI)	P>|z|	HR (complete)	P>|z|
Sex	1.35	0.002	1.36	0.005
Age	1.03	<0.001	1.03	<0.001
Ratio of LN at SNB vs LSG (≥1 vs <1)	0.88	0.21	0.91	0.078
Log(mitotic count)	1.21	0.002	1.22	0.001
Presence of ulceration	1.42	<0.001	1.54	<0.001
Breslow thickness	1.13	<0.001	1.14	<0.001

Abbreviations: HR = Hazard ratio; LN = Lymph node; MI = Multiple imputation; SNB = Sentinel LN biopsy

As can be seen in the above table, the hazard ratio for each variable is very similar between the 2 models using MI data and complete data only. This confirms that the multiple imputation process has not produced estimated missing values which are wildly abnormal.

Table 16 – Comparison between models using complete data only (complete) and using imputed missing data (MI) for disease-free survival

	HR (MI)	P>|z|	HR (complete)	P>|z|
Sex	1.22	0.011	1.16	0.09
LN location		0.0076*		0.012*
Lower limb vs upper limb	1.24	0.011	1.30	0.006
Axial vs upper limb	1.32	0.004	1.32	0.010
Multiple vs upper limb	1.04	0.805	1.03	0.865
Ratio of LN at SNB vs LSG (≥1 vs <1)	0.90	0.168	0.96	0.433
Log(mitotic count)	1.18	<0.001	1.23	<0.001
Breslow thickness	1.12	<0.001	1.14	<0.001

Abbreviations: HR = Hazard ratio; LN = Lymph node; MI = Multiple imputation; SNB = Sentinel LN biopsy.
*Overall P-value estimated by Wald test.

Again, for disease-free survival, the models using the different data are similar, although less so than for overall survival.

Interpretation of results for a non-statistical audience

Overall Survival

Hazard ratio is a way of conceptualizing the risk of "failure" – in this case, it represents the ratio of the risk of dying at any one time in one group of patients compared to another. The 95% confidence interval represents the range of hazard ratios that would be found in 95% (ie, 19 of 20) of studies if one were to repeat the study many times. That is, one is 95% confident that the true hazard ratio lies within the given range.

The final model for overall survival with hazard ratios, P-values and confidence intervals is shown in table 8. Note that this model is stratified according to the presence of positive

LN and the location of the sentinel LN. That is, the following results can only be interpreted by comparing patients with the same combination of the above two factors.

According to this final model, we found that:

- Male sex is associated with a 35% higher risk of death than females. Had we repeated the study 20 times, the increased risk will be found to be between 11% and 63% greater in men than women in 19 studies, on average.
- There is an increasing risk with increasing age. For 2 people with an age difference of 1 year, the risk is 3% higher for the older person. This will be found to be between 2-3% in 95% of times.
    - The hazard ratio for a 10-year age difference can also be calculated by:
        - $HR_{10y} = \exp(10b_{1y}) = \exp(0.272) = 1.31$
    - Therefore, in 2 people with an age difference of 10 years, the risk of death in the older person is 31% greater than that of the younger person. Similarly, the 95% confidence interval can be worked out to be 23% to 40%.
- The presence of ulceration is also an important factor, those patients with ulceration having a 42% higher risk of death at any time. We are 95% confident that this lies between 18 and 71%.
- The thicker the tumour, the higher the risk of death. For each 1mm increase in thickness, the risk of death is increased by 13%. We are 95% confident that the increase in the risk of death is between 8 and 17% for each 1mm increase in depth.
- In relation to the number of mitoses found at histology, for each logarithmic unit difference increase of mitoses, the risk of death increases by 21%. That is, for each 2.7 times increase in the number of mitoses detected on histopathology, the risk of

death increases by 21%. We can be 95% certain that this is between 7% and 37% increase in risk with increasing mitotic count.

- Finally, in relation to the outcome of interest, the lymph node yield was not found to have a statistically significant effect on survival. The P-value was 0.21, which means that there is a 21% chance that the observed magnitude of change could have occurred purely by chance alone. This is greater than our preset upper limit of 5% which we consider statistically significant. In the currently sampled patients, it seems that the presence of a high lymph node yield (>1) at surgery will lead to a 12% decrease of the risk of death. However if we repeated this study many times, 95% of times we will get a result that ranges from improving the risk of death by 28% to increasing the risk of death by 8% (95% CI 0.72-1.08%).

We therefore cannot conclude that a high lymph node yield at surgery (LN at SNB/LN at LSG>1) is associated with improved overall survival.

Disease-free survival

The final model for disease-free survival is detailed in table 13. This model is stratified by age, lymph node positive status, and presence of ulceration.

Therefore, this study shows that:

- Male sex is associated with a 22% higher risk of recurrence or death compared to females. Had we repeated the study 20 times, the increased risk would have been found to be between 5% and 43% greater in men than women in 19 times, on average.
- Compared to patients who have their sentinel nodes found in the upper limb nodes, those who have sentinel nodes found in the lower limb will have a 24% higher risk of recurrence/death. If we repeated this study many times, we would

find that this increase in risk lies between 5 and 47% in 95% of the times. Sentinel nodes being found in the neck or torso is also associated with an increased risk of recurrence or death of 32%. We are 95% confident that this increase in risk is between 9 and 58%.

- The thicker the tumour, the higher the risk of recurrence or death. For each 1mm increase in thickness, the risk of recurrence or death is increased by 12%. We are 95% confident that the increase in the risk of recurrence or death is between 8 and 16% for each 1mm increase in depth.
- In relation to the number of mitoses found at histology, for each logarithmic unit increase in mitoses, the risk of recurrence or death increases by 18%. That is, for each 2.7 times increase in the number of mitoses detected on histopathology, the risk of recurrence or death increases by 19%. We can be 95% certain that this is between 8% and 30% increase in risk with increasing mitotic count.
- Finally, in relation to the outcome of interest, the lymph node yield was not found to have a statistically significant effect on disease-free survival. The P-value was 0.17, which means that there is a 17% chance that the observed magnitude of change could have occurred by pure chance alone. This is greater than our preset upper limit of 5% which we consider statistically significant. However, on average, the presence of a high lymph node yield (>1) at surgery would lead to a 10% lower risk of recurrence/death. The 95% confidence interval in this case is between 24% decrease and 5% increase in risk of the same.

We therefore cannot conclude that a high lymph node yield at surgery (LN at SNB/LN at LSG>1) is associated with improved disease-free survival.

Discussion

Missing data

Missing data are almost inevitable in large datasets such as this one. Missing data can be classified into 3 categories[10]:

- Missing completely at random (MCAR) – these missing data are thought to arise completely randomly in that the property of "missingness" does not relate to any known information in the data but rather to extraneous factors (such as missing patient weight variable if that is due to malfunction of the scales on that visit.)
- Missing at random (MAR) – this kind of missing data is the most commonly assumed kind. Whilst the missing data arise apparently randomly, any systematic difference between missing values and observed values can be explained by differences in observed data. For instance, patients with poor outcomes may be less likely to return for follow-up, so these patients would have missing follow-up data.
- Not missing at random (NMAR) occurs when it is known that certain data are missing in a non-random fashion.

It is important to distinguish between these types of missing data as they affect the analysis.

There are many different ways of dealing with missing data. The most commonly used way to deal with incomplete data is either to exclude the subjects with missing data from the analysis or to use the indicator method. The former method may be acceptable if the data is indeed MCAR, however, it is often impossible to know that this is the case. This method will also generate unbiased estimates if the missing data are for:

- an outcome variable that is measured once in each individual
- a predictor variable that is unrelated to the outcome.

However, even in cases where such a method generates unbiased estimates, analysing the data this way will lead to a loss of precision and power.[10]

Another possible method of dealing with missing data is to use an "indicator method". In this method, an indicator variable is added as an extra level for each variable with missing data. The problem with this is that it inevitably causes bias and it also causes a large number of new variables.[11, 12]

The best way to deal with missing data is the use of multiple imputation.[10] The principle of this technique is based on the fact that MAR data are related to other variables, so their distribution can be estimated from the other measured variables. A new set of data can therefore be generated with the missing data thus estimated. Essentially, the estimated population distribution of the variables with missing values is re-sampled to create a new sample. As the imputed data and the actual data come from the same population distribution, modelling using the new completed dataset would yield non-biased estimates.

The variance of the estimates however, would be increased compared to modelling of the complete data. This additional variance from the "resampling" (imputation) process is minimised by combining the estimates from models of each of the imputed datasets, which is why multiple is preferable to single imputation.

Important issues that need to be considered in using this technique include:

- The number of imputations required: it is said that 5 imputations is usually adequate[8, 9]

- Selecting the variables used for analysis – as many variables should be used as possible, especially the outcome variable as it often carries information about the missing variable.[13]
- Non-normally distributed variables – these should be transformed to approximate normality before imputation.

Limitations of results

Limitations related to study design and data

Retrospective studies are often limited by the design and the data that are available for researchers. This is also the case in this study.

Being an observational study rather than a randomised controlled trial, there may be additional confounding factors which may not have been accounted for by the multivariate model. These unknown factors may lead to bias of the result.

One of the issues in this study is the short follow-up period after sentinel lymph node biopsy. Whilst the median follow-up was 46.5 months, median survival was not able to be estimated when calculating the overall survival. Median disease-free survival was just able to be calculated. However, the natural history of melanoma is that recurrences or even mortality do occur many years after the primary diagnosis. This may lead to inaccuracies in estimating median survival as there are few patients with more than 10 years of data.

Another limitation of the data arises from the accuracy of the collected data. In many cases, clinical databases are maintained by one individual, but the data are input by a variety of clinical and non-clinical staff. There is great variation in the accuracy of the data entered into the database depending on who enters the data. This is a potential source of bias, especially in a multi-clinician, multi-disciplinary institution such as the Sydney Melanoma Unit. If, for example, different clinicians with interests in different types of

melanomas (eg, metastatic or high grade) record data with different quality or completeness, then this will bias the data. However, this was partly ameliorated by the fact that consecutive patients were examined, and careful data cleaning was in place to review any inconsistencies in the data.

Another important limitation to consider is that the study setting in this case is at a specialist melanoma unit, which may see a different population of melanoma patients than that encountered in more general surgical/dermatology units. As sentinel node biopsy is most beneficial in patients who have intermediate thickness melanoma, this may affect the external applicability of this study.

However, one good point of the current study is that the sample size is relatively large, which will reduce the probability of making a type 2 error. This is important especially since we have found that lymph node ratio did not affect patient outcome. Note that this does not impact on the potential biasing effects of unrecognised confounders.

Limitations related to scientific methodology (lymphoscintigraphy)

As this study is retrospective one is unable to determine for certain whether all sentinel nodes have been removed, so we utilize the lymph node yield as a surrogate for this. A ratio of more than 1 does not necessarily reflect complete sentinel lymph node excision. Furthermore, lymphoscintigraphy is imperfect, which may also bias the outcome. The quality of lymphoscintigraphy is as important in this study as the quality of surgery.

Limitations relating to analysis

Whilst multiple imputation was used to estimate missing data, there are limitations to this technique. The first is the assumption that the missing data are MAR. If there were data that were NMAR, then the imputed missing values would be biased. Also, imputing

values of variables with a non-normal distribution may lead to imputation of extreme or impossible values which again may lead to bias.

Finally, when multiple imputation is used with modelling, it is not clear whether the values should be imputed before the model-building process or if it should be performed after. The former may lead to bias by not incorporating into the imputation process what can only be known after modelling (eg, non-linear functional forms of continuous covariates) whilst the latter can lead to bias by restricting the model-building process to observations with complete data.[8]

In this study, we have attempted to evaluate the effect of multiple imputation by comparing the resulting analysis with an analysis restricted to complete data only. The results confirm that multiple imputation has not led to very abnormal results in this study. The discrepancies seen between the two analyses probably reflect the bias due to restricting analysis to observations with complete data only.

Conclusion

Complete extraction of all sentinel lymph nodes identified at preoperative lymphoscintigraphy was not found to be associated with statistically significant improvements in overall or disease-free survival.

Other well-known risk factors (male sex, older age, presence of involved nodes, presence of ulceration, increased Breslow thickness, high mitotic count, and location of tumour) were once again confirmed to affect outcome.

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Appendix – Excel data manipulation

Note: **DATA** signifies original data input by data collector/investigator.

Data table 1

To code sex (Column D – "Code M/F")

	C	D
1	M/F	**Code M/F**
2	**DATA – eg, "M", "F"**	=IF(C2="M",1,IF(C2="F",0,"error"))

To calculate age:

	E	F	G
1	Date of Birth	Date of SNB	**Age**
2	**DATA – in form of date**	**DATA – in form of date**	=(F2-E2)/365.25

To code ulceration (Column AU – "Ulcer code")

	AT	AU
1	Ulceration	**Ulcer code**
2	**DATA – "Nil" or "Yes"**	=IF(AT2="Nil",0,IF(AT2="Yes",1, IF(AT2="","","error")))

To code side (Column J – “Left or right”) and area of lymph node basin (Column T – “Final LN Area”)

	I	J	K	L	M	N	O	P	Q	R	S	T
1	Area of lymph node	**Left or Right**	Lnarea	Groin	Popliteal	Axilla	Epitrochlear	Triangular space	Neck	Other	Reclass	**Final LN area**
2	**DATA, eg, Rgroin, Laxilla**	=IF(COUNTA(I2)=1,IF(ISERR(FIND("R",I2)=TRUE),IF(ISERR(FIND("L",I2))=TRUE,"error",IF(FIND("L",I2)=1,"0","2")),IF(FIND("R",I2)=1,"1","2")),"")	=IF(COUNTA(I2)=1,IF(ISERR(FIND("/",I2))=TRUE,IF(ISERR(FIND("axilla",I2))=TRUE,IF(ISERR(FIND("groin",I2))=TRUE,IF(ISERR(FIND("neck",I2))=TRUE,"error",3),2),1),4),"")	=IF($K2=4,IF(ISERR(FIND("groin",$I2))=FALSE,TRUE,FALSE),"")	=IF($K2=4,IF(ISERR(FIND("pop",$I2))=FALSE,TRUE,FALSE),"")	=IF($K2=4,IF(ISERR(FIND("axil",$I2))=FALSE,TRUE,FALSE),"")	=IF($K2=4,IF(ISERR(FIND("epit",$I2))=FALSE,TRUE,FALSE),"")	=IF($K2=4,IF(ISERR(FIND("triang",$I2))=FALSE,TRUE,FALSE),"")	=IF($K2=4,IF(ISERR(FIND("neck",$I2))=FALSE,TRUE,FALSE),"")	=IF($K2=4,IF(OR(L2,M2,N2,O2,P2,Q2)=TRUE,"",1),"")	=IF(K2=4,IF(AND(OR(L2,M2),NOT(OR(N2,O2,P2,Q2))),2,IF(AND(OR(N2,O2,P2),NOT(OR(M2,L2,Q2))),1,IF(AND(Q2,NOT(OR(P2,O2,N2,M2,L2))),3,4))),"")	=IF(K2=4,S2,K2)

To extract number of positive lymph node (Column Y – “NoPos”)

	X	Y
1	Histology	**NoPos**
2	**DATA, in the form of: “0\2”, “1\2” etc**	=IF(COUNTA(X2)=1,MID(X2,1,FIND("\",X2,1)-1),"")

To extract tumour type code (Column AR – “Type code”) and thickness (Column AS – “Thickness”) from original data input (Column AP)

	AP	AQ	AR	AS
1	Type, Thickness	Type	**Type code**	**Thickness**
2	**DATA, in the form of “SSM 1.3”, “NM 2.9”, etc**	=IF(COUNTA(AP2)=1,IF(COUNT(AP2)=1,"",MID(AP2,1,FIND(" ",AP2,1)-1)),"")	=IF(AQ2="","",IF(AQ2="SSM",1,IF(OR(AQ2="SSM/NM",AQ2="NM"),2,IF(AQ2="AL",3,IF(AQ2="LM",4,IF(AQ2="Des",5,IF(OR(AQ2="MIS",AQ2="Blue"),9,"error"))))))	=IF(COUNTA(AP2)=1,IF(COUNT(AP2)=1,AP2,MID(AP2,FIND(" ",AP2,1)+1,4)),"")

Table 2 – Events datasheet – working out a list of dates of distant recurrences

	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	PIN	…	…	…	…	Date of SNB			← This shaded area is a copy of a limited area of the main datasheet (datasheet 1). This is simply to supply a list of SNB dates for lookup in the main data area below								
2	741					2/1/2001											
3128	…	…	…	…	…	…											
3132		PIN locoregional recurrence	PIN distant recurrence	PIN any recurrence	SNB date	Event date	Event type	Event description	Recurrence						Event description 2	Local/regional	distant
3133		=IF(AND(J3132=1,Q3132=1,G3132>F3132),B3132,"")	=IF(AND(J3132=1,R3132=1,G3132>F3132),B3132,"")	=IF(AND(J3132=1,G3132>F3132),B3132,"")	=VLOOKUP(B3132,$A$2:$G$3128,7,FALSE)	**DATA – in form of date**	**DATA – in form of event colde**	**DATA – eg, "Treatment", "Recurrence"**	=IF(I3132="Recurrence",1,0)	=IF(I3132="Date of birth",1,0)	=IF(I3132="Primary Melanoma",1,0)	=IF(I3132="Treatment",1,0)	=IF(I3132="Last status",1,0)	=SUM(J3132:N3132)	**DATA – eg, "Surgery: local" or "Dead melanoma"**	=IF(J3132=1,IF(OR(NOT(ISERR(FIND("Regional",P3132)))=TRUE,NOT(ISERR(FIND("Local",P3132)))=TRUE,NOT(ISERR(FIND("Iocoregional",P3132)))=TRUE,NOT(ISERR(FIND("Extracapsular",P3132)))=TRUE,NOT(ISERR(FIND("transit",P3132)))=TRUE),1,""),"")	=IF(J3132=1,IF(ISERR(FIND("Distant",P3132))=FALSE,1,""),"")

...	...	...	...	...	...												
29140	PIN	4	6	13	7		← This shaded area of the data table is the output area. It was then copied to the main datasheet in Excel (Sheet 1), then to Stata. This represents a table with a list of PINs, date of distant recurrence, and a censoring variable with 1 representing the occurrence of distant metastasis. A similar table was produced on a separate worksheet for locoregional recurrence.										
29141	741	=IF(ISNA(VLOOKUP($B29140,$D$3131:$Q$29122,C$29139,FALSE))=TRUE,"",VLOOKUP($B29140,$D$3131:$Q$29122,C$29139,FALSE))	=IF(ISNA(VLOOKUP($B29140,$D$3131:$Q$29122,D$29139,FALSE))=TRUE,"",VLOOKUP($B29140,$D$3131:$Q$29122,D$29139,FALSE))	=IF(ISNA(VLOOKUP($B29140,$D$3131:$Q$29122,E$29139,FALSE))=TRUE,"",VLOOKUP($B29140,$D$3131:$Q$29122,E$29139,FALSE))	=IF(ISNA(VLOOKUP($B29140,$D$3131:$Q$29122,F$29139,FALSE))=TRUE,"",VLOOKUP($B29140,$D$3131:$Q$29122,F$29139,FALSE))											
	...	...	...	...	...												