Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Carrie Kai-Cheng Chu Date

The Role of Age in Sentinel Lymph Node Radioactivity and Metastasis in Melanoma

By

Carrie Kai-Cheng Chu
Master of Science

Clinical Research

Theresa Gillespie, Ph.D. Advisor	John Boring, Ph.D. Committee Member
David Kooby, M.D. Advisor	John McGowan, M.D. Committee Member

Thomas Ziegler, M.D.
Committee Member

Accepted:

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

Date

The Role of Age in Sentinel Lymph Node Radioactivity and Metastasis in Melanoma

By

Carrie Kai-Cheng Chu
M.D., Georgetown University, 2005
B.A., Cornell University, 2001

Advisor: Theresa Gillespie, Ph.D.
Advisor: David Kooby, M.D.

An abstract of
A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science
in Clinical Research
2010

ABSTRACT

The Role of Age in Sentinel Lymph Node Radioactivity and Metastasis in Melanoma
By Carrie Kai-Cheng Chu

Background: Elderly patients have less favorable melanoma-specific survival in comparison to the young, despite reduced risk of nodal metastasis. Age-dependent decline in lymphatic function is a postulated explanation.
Hypothesis: Lymphatic function and odds of sentinel lymph node (SLN) metastasis are statistically reduced in melanoma patients with older age compared to their younger counterparts, controlling for other patient, disease, and technical variables.
Study Design: For this cross-sectional study, a prospectively-maintained institutional melanoma database was queried for cases of lymphoscintigraphic mapping and SLN biopsy from 1994-2008. Data regarding highest ex-vivo SLN radioactivity as measured during biopsy were gathered and analyzed as a measure of lymphatic function. Associations of age with SLN radioactivity and metastases were assessed using linear and logistic regression.
Results: Radioactivity counts were available in 1349 patients. Age correlated negatively with radioactivity count ($r= -0.164$, $p<0.001$). Head/neck primaries and cervical SLN were associated with reduced counts, while ulceration, increased radiocolloid dose, and increased SLN number were associated with elevated counts. On multivariate analysis, increasing age was the strongest independent predictor of reduced radioactivity ($\beta= -109.9$, $p<0.001$). Colloid dose ($\beta= +2.3$, $p=0.02$), SLN number ($\beta= +622.9$, $p<0.001$), ulceration ($\beta= +1596.7$, $p=0.02$), and cervical SLN ($\beta= -2265.2$, $p=0.01$) also maintained independent association with count variability. SLN metastasis was detected in 17.9% of all patients. Prevalence of SLN disease declined with increasing patient age ($p=0.02$). On multivariate analysis, increasing age (odds ratio 0.98, 95% confidence interval [0.97-0.99], $p<0.001$) and head/neck primary site (odds ratio 0.57, [0.34-0.57], $p=0.03$) were negative predictors of SLN disease.
Conclusions: Increasing age is an independent predictor of declining lymphatic function as measured by SLN radioactivity counts, paralleling an age-related reduction in odds of SLN metastasis. Age-related lymphatic dysfunction is a potential explanation for the paradoxical increase in mortality despite reduction in SLN disease in elderly patients with melanoma.

The Role of Age in Sentinel Lymph Node Radioactivity and Metastasis in Melanoma

By

Carrie Kai-Cheng Chu
M.D., Georgetown University, 2005
B.A., Cornell University, 2001

Advisor: Theresa Gillespie, Ph.D.
Advisor: David Kooby, M.D.

A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science
in Clinical Research
2010

TABLE OF CONTENTS

INTRODUCTION

Over the past two decades, lymphatic mapping and sentinel lymph node (SLN) biopsy have gained widespread acceptance as a minimally-invasive tool to provide accurate staging and survival prognostication in patients with melanoma.(1, 2) In fact, regional nodal disease status has been established as the most critical predictor of survival in melanoma.(3, 4) When considering other determinants of melanoma-specific mortality, however, increasing age also independently confers decreased survival.(4, 5) The etiology behind this association is unclear, and particularly puzzling in light of a paradoxical age-associated decline in SLN metastasis that has been previously described.(5-8) The mechanism underlying the reduction in risk of SLN disease in elderly melanoma patients remains unknown.

Amongst various postulated but untested theories is the proposal that age-related anatomic and functional lymphatic deterioration leads to reduction in transit of tumor cells from primary sites to the regional nodal basins.(6) Although morphologic and physiologic lymphatic changes have been indirectly linked to aging in animal and human cadaveric studies,(9-14) the relationship between aging and lymphatic function in the context of melanoma metastasis has undergone limited evaluation.

Lack of standard measures of lymphatic function has represented one barrier to understanding of the relationship between age and lymphatic tumor dissemination. Increasing use of SLN biopsy in melanoma provides a unique means of quantifying lymphatic activity this population. Successful SLN localization is dependent upon diffusion of injected peri-tumoral radiolabeled colloid into the lymphatic plexus, subsequent transit via afferent channels, and eventual uptake into regional nodes.(15)

Intraoperative accumulated nodal radioactivity counts are routinely obtained for identification and biopsy of SLNs, but the utility of radioactivity counts as measures of lymphatic function is seldom recognized.(16)

This study aimed primarily to examine the relationship between age and lymphatic function as measured by accumulated SLN radioactivity in patients with melanoma, and secondarily to evaluate the corresponding age-related odds of SLN metastasis. We retrospectively collected data pertaining to patient characteristics, tumor features, and technical variables in 1,349 patients with primary cutaneous melanoma who received SLN biopsy at a single institution over a 15-year period. This cross-sectional study first examined the association of age at SLN biopsy with measured SLN radioactivity using multivariate linear regression with adjustment for patient, tumor, and technical variables. Subsequently, the effect of age on odds of SLN metastatic disease was modeled using multivariate logistic regression.

BACKGROUND

Epidemiology of Cutaneous Melanoma

In the United States today, melanoma ranks as the fifth most common cancer in men and the seventh most common amongst women.(17) The American Cancer Society reported that approximately 68,720 new cases of melanoma would be diagnosed in this country in 2009.(17) This estimate reflects a dramatic rise in the incidence rate of melanoma, which has more than doubled since 1973. The rate of increase is particularly marked in elderly patients. In patients aged 65 or greater, the incidence rate has steadily risen by 5.1% annually since 1985 amongst men, and by 4.1% annually since 1975 amongst women.

Despite refinements in diagnostics, surgical treatment, and systemic therapy, the mortality rate for melanoma has remained largely unchanged over the last ten years. For older patients, melanoma-specific mortality rate has actually risen since the 1990s. Nodal disease status is the most important predictor of outcome in this disease; ten-year survival for node-positive patients is approximately 24-59% as compared with 57-95% in patients who are node-negative.(18)

Rationale & Physiology of Sentinel Lymph Node Biopsy

Presently, nodal disease is most frequently detected via lymphatic mapping and SLN biopsy. Based on the concept of lymph drainage from peripheral tissues via an ordered, sequential anatomic process, the procedure identifies the first ("sentinel") node receiving drainage material from the primary tumor site.(1, 19) The SLN is thus the most likely site within its nodal basin to harbor metastatic tumor cells. In addition to accurate

staging and prognostication, results of SLN biopsy provide indications for further treatments including more extensive nodal dissection, regional chemotherapeutic infusion, and systemic immunotherapy.(20)

Following peri-tumoral injection, radioactive colloid initially enters the dermal terminal lymphatic capillaries, which lack intraluminal valves, complete basement membranes, and intact muscularis layers.(15) Entrance occurs via passive diffusion through overlapping endothelial cells containing 10-25 nm gaps that can be widened by massage or exercise, maneuvers which increase the volume and flow of lymph.(15) The rate of propulsion through subsequent valved afferent channels varies by anatomic location, with head/neck, upper extremities, and lower limbs representing ascending order of flow velocity.(21) Nodal entrapment of radiocolloid occurs via a complex process involving opsonization and phagocytosis by macrophages and histiocytes lining the sinuses.(22) Most of the radiocolloid that reaches a SLN will be retained in the node by the process. A small portion of the tracer can pass to second-tier nodes. The likelihood of passage to second-tier nodes is directly proportional to the rate of flow.(23)

A delayed radiographic lymphoscintigram is obtained following tracer injection to provide general guidance toward the appropriate anatomic basin of SLNs. In the operating suite, the surgeon uses a radioactivity detection device to identify individual SLNs for excisional biopsy. In addition, the surgeon may choose to perform peri-tumoral injection with blue dye; subsequent dye uptake by SLNs facilitates their visualization. This adjunctive practice is advocated to enhance sensitivity of the SLN biopsy procedure.(24)

SLN Radioactivity

During SLN biopsy, accumulated radiocolloid in the SLNs is typically detected using a hand-held gamma-probe.(25) Once the node is identified and removed, the surgeon places the probe in direct contact with the SLN in order to obtain a cumulative 10-second ex-vivo radioactivity count. This number is documented in the operative record. Further identification and retrieval of additional SLNs is continued until the residual radioactivity is <10% of the highest detected ex-vivo SLN radioactivity count.(25, 26)

Gamma-probe-detected counts measure the amount of radioactivity in SLNs.(27) SLN radioactivity occurs as a result of intranodal radiocolloid accumulation, which in turn requires colloid diffusion into lymphatic capillaries, transport via afferent channels, and uptake by lymph nodes.(15, 25) Count rates thus represent a quantified reflection of these processes as components of lymphatic function. Variability in SLN radioactive count rates may be attributable to patient, procedural, and pathologic parameters. In addition to age, medical history (prior surgery, radiation, or infection) may contain potentially influential patient variables.(28) Procedural determinants may include timing, dose, volume, type, and size of the injection agent, as well as type of detection probe.(29) Pathologic characteristics of primary lesions and SLN tumor burden may also contribute.(25, 30)

Age & Melanoma

Amongst elderly patients with melanoma, an incongruous observation of worse prognosis despite reduced SLN involvement exists in comparison with the young.(5)

Beyond decline in SLN disease, primary lesions in older patients have been characterized as having less favorable histologic characteristics such as increased Breslow thickness and mitotic figures.(6) Age-associated alterations in lymphatic anatomy and physiology are thought to contribute to alternative dissemination pathways and biologic behavior in melanoma,(6, 16) but the influences of age on decline in lymphatic function and risk of SLN disease have never been jointly demonstrated. The implications of aging on melanoma biology are poorly understood.

Age & the Lymphatic System

Knowledge regarding the effect of aging on the processes underlying successfully SLN biopsy is limited. Animal models have exhibited age-related impairment of lymphatic vessels dynamics.(12, 13) In humans, morphologic deterioration of the elastic elements and muscularis layers of lymphatic vessels are described with advanced age,(31) while fibrotic and fatty replacements of nodal parenchyma also occur.(14, 32) Mortimer et al found that lymphatic function as measured by quantitative lymphoscintigraphy declined with rising age in both patients with chronic leg ulceration and normal controls.(33) There is no current evidence suggesting reduced sensitivity of this SLN biopsy in elderly patients; increased age is not associated with greater risk of delayed nodal recurrence in patients with negative SLN biopsies.(6)

In 2002, Cox et al reported an inverse relationship between accumulated radioactive SLN counts and age in 1356 patients with breast cancer.(28) Age-dependent decline in SLN radioactive count rates in melanoma was first described by Conway et al in 2009.(16) In their retrospective review of 858 patients, increasing age was correlated

with diminishing mean ex-vivo one-second hottest SLN counts, independent of the site of the SLN basin.(16) Use of single-second counts, however, is associated with great measurement variability. Additionally, the impact of age on risk of SLN disease was not examined.

METHODS

Null Hypothesis:

-Primary Aim: Lymphatic function as measured by highest ex-vivo ten-second SLN radioactivity count does not differ statistically with increasing patient age at SLN biopsy for melanoma, adjusted for patient, disease, and technical variables,

-Secondary Aim: Odds of SLN metastatic disease does not differ statistically with increasing patient age at SLN biopsy, adjusted for patient, disease, and technical variables.

Study Design:

Retrospective cross-sectional study analyzed as a cohort study

Patients:

Patients were identified from an Emory University Institution Review Board-approved melanoma database that is prospectively-maintained by the Division of Surgical Oncology.

Inclusion Criteria:

1. Patient with histologic diagnosis of primary cutaneous melanoma.
2. Patient underwent successful lymphatic mapping with SLN biopsy at Emory University-affiliated hospitals from 1994 to 2008.
3. Documented ten-second ex-vivo highest SLN radioactivity count.

Exclusion Criteria:

1. Patients with pure desmoplastic melanoma subtype. Pure desmoplastic histology is a rare melanoma subtype associated with unique biologic profile including drastically reduced propensity for nodal metastasis.(7)

2. Patients with missing data pertaining to histopathologic variables of the primary tumor and SLNs, or technical variables related to the lymphatic mapping and SLN biopsy procedure.

Predictor Variables:

The primary predictor variable of interest was age in years at time of SLN biopsy. Age was examined in both continuous (years) and categorized (<30 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years, and ≥70 years) variable formats.

Additional predictor variables were related to patient, disease, and SLN biopsy technique. Besides age, patient gender was also considered. Primary disease-related variables included primary tumor location (head & neck, trunk, upper extremities, or lower extremities), Breslow thickness (mm), histologic evidence of ulceration or regression (present or absent), and histologic subtype (superficial spreading, nodular, lentigo maligna, acral lentiginous, other, and unclassified). Nodal disease-related variables included pathologic status (tumor present or absent) of both the most radioactive ("hottest") SLN and overall SLNs, hottest SLN basin (cervical, axillary, inguinal, in-transit), multiplicity of SLN drainage basins (single or multiple), and total number of procured SLNs. Technical variables included dose of radiocolloid injection (μCi), timing of injection relative to SLN biopsy (same-day, previous-day), and type of

radioactivity detection device used (before 2004: C-Trak® device, 2004 and after: Node Seeker™ device).

Outcome Variables:

Corresponding to the primary and secondary aims, there were two major outcome variables of interest. The highest ex-vivo SLN radioactivity (counts per 10 seconds) as a measure of overall lymphatic function was analyzed as a continuous variable. The utility of measured SLN radioactivity in this capacity, outside of its intended role as a guide for nodal excision during SLN biopsy, is seldom recognized. Due to lack of gold standards in assessment of lymphatic function, the predictive value of radioactivity counts as a measure of lymphatic function is unknown. However, rates of injected radioisotope transport and uptake in regional basins calculated via quantitative lymphoscintigraphy are well-established, *non-invasive* metrics of lymphatic function in studies of healthy and lymphedema patients.(33, 34) Contact probe-detected nodal radioactivity is less frequently used due to the invasive nature of node exposure, but access to ex-vivo nodal tissue afforded by SLN biopsy allows for direct measurement of accumulated nodal radioactivity as, at minimum, quantitative surrogates of a component of lymphatic function.(22, 35) For this analysis, individual chart review was conducted retrospectively to collect SLN radioactivity data from qualifying patients identified from the prospectively-maintained database.

Metastatic invasion of SLNs (positive or negative) as derived from surgical pathology reports was the second outcome of interest. Because overall SLN pathologic status, in contrast to hottest SLN pathologic status, is the known determinant of

melanoma-specific survival, patients with disease involvement of any biopsied SLN were considered to be SLN-positive. SLN biopsy is a well-established method for detection of nodal disease in melanoma, with reported sensitivities of 87-90% and specificity of 100%.(36, 37) False negatives in such studies are typically identified as patients with negative SLN biopsy who develop subsequent regional nodal recurrence in the sampled basin.

Analysis:

Measures of frequency were calculated and presented as means ± standard deviation for continuous variables or proportions/percentages for categorical variables. Linear association between age (continuous variable) and highest SLN radioactivity was examined using Pearson's correlation coefficient with test for significance. Correlations between SLN radioactivity and other predictor variables were similarly examined. Age categories as previously defined were treated as ordinal variables for comparison of mean highest SLN radioactivity per age category; one-way analysis of variance (ANOVA) was used to test for significance of differences in group means.

Based on a priori knowledge from existing literature or clinical plausibility from subject matter knowledge, age and other predictor variables were entered into multiple linear regression models to estimate their adjusted effect on variability in highest SLN radioactivity. Outlier checking using Cook's distance, Leverage statistics, and Jackknife residuals was performed. Although each method identified potential outliers, subsequent data verification did not demonstrate any biologically implausible or definitively erroneous information. Assessment for multicollinearity amongst predictor variables using variance inflation factors indicated no severe collinearity (all factors <10). Residuals versus

predicted value and normal probability plots of the residuals showed no violation of linearity or homoscedasticity. No gross assumption violations were identified for linear regression analysis. Analysis for interaction was carried out by inclusion of all possible two-way interaction terms with subsequent backward elimination for significant interaction terms using the Wald test. No significant interaction terms were identified, and further comparison of the full (all two-way interactions) and the no-interaction models using the F statistic demonstrated no significant differences.

Forward, backward, and step-wise covariate selection strategies were used with convergence of methods into a model of maximal parsimony while maintaining clinical relevance. Results are presented as estimated correlation coefficients (estimated β), standard errors, and corresponding p-values.

For the second portion of the study, proportions of patients with SLN disease across ascending age categories were compared using the chi-squared test for trend. SLN-positive and SLN-negative patients were compared with respect to additional clinicopathologic predictor variables using Pearson chi-squared or pooled independent-sample t-tests as appropriate.

For age and other predictors, adjusted odds of SLN metastasis were modeled using binary logistic regression. Covariate selection was based on *a priori* subject matter knowledge. For categorical predictor variables with greater than two levels, dummy variables were created. Assessments for multicollinearity and statistical interaction were carried out as described above (likelihood ratio test for comparison of full and reduced models). No significant interaction terms were identified. Evaluation of confounding of the hypothesized association between age and SLN disease was performed by

comparison of crude and adjusted odds ratios of SLN disease for age with inclusion of the variable of interest. Additionally, clinically-recognized determinants of SLN disease (such as tumor ulceration and depth) were included. Hosmer-Lemeshow test was used to assess the overall goodness of fit of the final model. Results are reported as odds ratio estimates with 95% confidence intervals.

Alpha level was set at 0.05 for all statistical tests, and all reported p-values were two-tailed where appropriate. Analysis was performed using SAS version 9.2 for Windows. Figures were generated using SPSS version 17.0 for Windows.

Clinical Procedures:

Lymphoscintigraphy

Patients underwent cutaneous lymphoscintigraphy using filtered (0.22 micron) 99mTc-labelled sulfur colloid (Pharmalucence, Inc., Bedford, MA) on the day of SLN biopsy. Rarely, lymphoscintigraphy took place one day prior. Four intradermal radiocolloid injections were placed circumferentially around the primary lesion or biopsy site; injection dose (μCi) was recorded. Dynamic lymphoscintigraphy was performed with planar gamma camera imaging every 10 seconds for 10 minutes to identify focal areas of accumulation followed by multiple 5-minute static images up to 60 minutes. Two-hour post-injection delayed images were obtained in some patients.

SLN Biopsy

Intra-operative vital blue dye injection was used routinely in the latter half of the study. Measurement of radioactive count rates in the radiolabeled nodes were made intraoperatively using a handheld gamma probe (before 2004: C-Trak®, Care Wise, Morgan Hill, CA; 2004 and after: Node Seeker^{TM}, Intramedical Imaging LLC, Los

Angeles, CA). SLNs demonstrated increased focal radiotracer uptake (hot spots). Areas of increased activity were removed, and individual nodes were dissected out to assess focal radiotracer uptake. Ex-vivo counts were accumulated over 10 seconds and recorded. Lymphoscintigrams were used to guide the number of removed SLNs. SLNs were removed until the 10-second lymphatic bed count was approximately 10% of the hottest SLN count. Any additional blue nodes or un-dyed nodes accompanied by blue channels were also removed. Nodal basins were stratified by location (inguinal, axillary, and cervical). In-transit SLNs were exclusive of the main basins and included epitrochlear, popliteal, and parascapular nodes.

Institutional practices for histopathologic processing and evaluation of retrieved SLN have been previously described in greater detail.(36) In general, retrieved SLN were bisected parallel to the long axis and both halves entirely submitted for hematoxylin-and-eosin, S100, and HMB-45 staining. SLN <5mm were completely embedded while those >10 mm were divided into >2 pieces at 2-3 mm intervals prior to embedding.

RESULTS

Patients

Between 1994 and 2008, 1349 patients underwent successful SLN biopsy for cutaneous melanoma and had sufficient data for analysis. Mean age was 52.1 years, with a minority of female patients (40.5%). Table 1 summarizes overall tumor-related characteristics.

Radioactivity Count Rates

The decline in mean hottest radioactive count rates across ascending age categories ($p<0.001$) is illustrated in Figure 1. Age as a continuous variable likewise correlated inversely with count rates ($r= -0.16$, $p<0.001$). Age-related rise in prevalence of male patients, head/neck primaries, primary ulceration, and cervical drainage is summarized in Table 2. Older patients on average also presented with thicker primaries (Table 2). Dose of radiocolloid, multiplicity of drainage, and number of removed SLN were similar across age groups (Table 2).

On bivariate analysis as shown in Table 3, lower mean radioactive count rates were observed with head/neck primaries, cervical nodal basins, and in-transit drainage. Patients without primary ulceration and those undergoing prior-day colloid injections also had lower mean counts, though the differences were not statistically-significant. In contrast, dose of radiocolloid injection and number of SLNs removed were both positively correlated with count rates. Gender, Breslow depth, number of basins, and SLN pathology appeared to be unrelated to count rates. The final multiple linear regression model estimated a 110 per 10-second decline in highest radioactive SLN count

rate with each year progression in age (β= -109.9, p<0.001). Relative to inguinal drainage, cervical basins also demonstrated an independent effect on count rates (β= -883.6, p=0.013); additional parameter estimates by drainage site are reported in Table 3. Other positive predictors of radioactive counts were presence of ulceration (β= +1596.7, p=0.02, higher radiocolloid dose (β= +2.7, p=0.02), and increasing number of SLNs (β= +622.9, p<0.001) (Table 3).

Nine-hundred thirty-three (69.2%) patients underwent SLN biopsy prior to 2004, the year when detection probes were replaced. To account for associated differences in radiosensitivity, patients were stratified based on year of SLN biopsy. Mean patient ages were similar (before 2004, 52.1 years; 2004 and after, 52.2 years, p=0.97), while mean hottest counts were significantly higher using the newer probe (before 2004, 6724 per 10 seconds; 2004 and after, 11100 per 10 seconds, p<0.001). Within each stratum, application of the same regression model showed that increasing age maintained independent association with reduced radioactivity (before 2004, β= -92.8, p<0.001; 2004 and after, β= -168.6, p<0.001).

SLN Disease

SLN metastases were identified in 242 patients (17.9%). Compared to those with SLN disease, patients without SLN involvement were significantly older (SLN-negative 52.6 ±15.0 years, SLN-positive 50.2 ±15.6, p=0.026). Additional comparisons of patient- and tumor-related features are shown in Table 4. Detection of SLN disease was independent of radiocolloid dose (SLN-negative 338.8 ±303.0 μCi, SLN-positive 330.5 ±238.2, p=0.70).

Figure 1 demonstrates the decline in unadjusted prevalence of SLN disease across increasing age categories ($p=0.02$). Adjusted for sex, primary site, Breslow depth, and presence of ulceration, the odds of SLN disease declined by 2% with each increasing year of age (odds ratio 0.98, 95% confidence interval 0.97-0.99, $p<0.001$). Additional predictors of SLN metastasis are shown in the logistic regression model in Table 5.

DISCUSSION

The paradoxical decline in risk of SLN metastasis with increasing patient age has been previously attributed to age-dependent decline in lymphatic function. The purpose of this study was to examine the relationship between age and lymphatic activity as measured by SLN radioactivity during biopsy for melanoma, with subsequent estimation of the corresponding age-related odds of SLN disease. Increasing age was independently associated with decline in highest SLN radioactivity as well as odds of SLN metastasis, suggesting that age-related deterioration of lymphatic function may explain in part the observed decrease in SLN melanoma involvement in older patients.

In addition to increasing age, cervical SLNs were also associated with reduced radioactivity. This finding is consistent with existing knowledge regarding the increased variability of lymphatic structure and low-velocity lymphatic flow in the head and neck region relative to other anatomic sites.(21, 25, 38) More importantly, although prevalence of head and neck melanomas is increased amongst older patients, the effect of increasing age on SLN radioactivity was observed independently of the effect of cervical SLN location.

Only one study by Conway and colleagues in 2009 has previously examined the relationship between age and SLN radioactivity in melanoma.(16) The current analysis using data from 1349 patients represents the largest published study regarding this association, and further demonstrates the adjusted age-dependent decline in odds of SLN disease in the same group of patients. Beyond sample size, the current study also draws strength from its use of cumulative 10-second ex-vivo SLN radioactivity counts, a reproducible measure with less variability compared with 1-second counts. Additionally,

we demonstrate a consistent dose-dependent relationship between increasing age and both of our outcomes of interest (radioactivity and SLN disease). Despite the cross-sectional nature of the data used, by its own definition age at melanoma diagnosis (or SLN biopsy) temporally precedes the SLN procedure, thereby validating consideration of age as a predictor variable of the two main outcome variables in similar fashion to a cohort study. Additionally, the single-institution nature of this study afforded uniformity in method of SLN radioactivity measurement.

However, several limitations warrant consideration. First, despite adjustment for theorized confounders, the observed independent association between age and SLN radioactivity cannot be concluded as causal based on our analysis. The retrospective nature of the study design precluded consideration of either additional unrecognized confounders or recognized but unmeasured confounders. For example, acquisition of medical comorbidities and receipt of surgeries that may interfere with lymphatic drainage likely accumulate with age. Definition of relevant medical conditions and surgical procedures, along with their timing with respect to SLN biopsy is, however, difficult within the confines of the current study design. Arguably, if a causal association of age with SLN radioactivity is mediated via illness and surgeries, age at diagnosis is a more objective variable in the causal chain. In reality, however, the mechanism of association remains undefined.

As was evident during statistical analysis, despite consideration of several patient-, disease-, and technique-related variables, a considerable amount of variability in SLN radioactivity remained unexplained. Unmeasured determinants of variability include the actual volume of radiocolloid injection (as opposed to dose) and, as a related factor, the

concentration of the agent.(39, 40) The injection agent is prepared daily by nuclear medicine technicians, and the degree of variability between days and individual technicians despite existence of a standardized mixing protocol cannot be adequately accounted for. Variation may also exist in the amount of elapsed time between injection and SLN biopsy on the order of hours (in contrast to the days between injection and biopsy), thus influencing detected radioactivity. However, there is no clinical reason to suspect unequal distribution of patient age with respect to these technical variables. While their measurement and inclusion in a prospective fashion may improve model predictive ability, omission of these factors is not expected to distort the observed association between age and SLN radioactivity.

Finally, performance of ex-vivo SLN radioactivity as a test for overall lymphatic activity is not well-validated. As previously discussed, lack of gold standards in measurement of lymphatic function precludes rigorous definition of the sensitivity and specificity of lymphatic radioactivity in reflection of overall lymphatic function. Further, the ability to obtain direct node-contact gamma probe counts is unique to the invasive context of excisional nodal biopsy, and would be otherwise not indicated in healthy individuals. Because successful detection of radioactivity is dependent upon the transport and uptake of injected molecules into regional nodes, however, the measurement is necessarily a function of overall lymphatic activity. Although beyond the scope of this study, some previous literature suggests that failure of tracer migration and uptake and, thus, SLN identification, occurs more frequently with increasing age, an observation consistent with the current framework. Evaluation of SLN radioactivity and overall lymphatic function at a population level would be difficult, as administrative

databases do not and are unlikely in the future to incorporate the relevant and necessary technical data.

Despite these limitations, the possibility of a prospective investigation at the multi-institutional level remains promising in light of our findings. This study provides indirect support of age-related lymphatic function decline as a theorized explanation for decrease in risk of nodal melanoma disease with rising age. Whether the decline occurs as a result of anatomic deterioration or other pathophysiologic processes warrants further study. In a prospective setting, it may be useful to obtain concurrent sampling of lymphatic channels and other peri-lymphatic, subcutaneous tissue during SLN biopsy in order to correlate histopathologic findings with age. It has also been suggested that the biology of melanoma in elderly patients may differ from that amongst their younger counterparts, and investigation of tumor lymphangiogenesis as influenced by age may be elucidating. For example, vascular endothelial growth factor activation by melanoma with subsequent increased propensity for nodal dissemination is of late receiving much attention; activation with respect to age may be an area for investigation.(41) The role of individual and tumor genetic profile represents another source of potential explanation.

The unfavorable melanoma-specific survival amongst the elderly relative to younger patients remains unexplained, as mortality was not evaluated in this study. Future studies of disease recurrence patterns as related to age at diagnosis may be useful; such studies using population-based data are increasingly feasible given the growing availability of large, validated cancer databases such as the Surveillance Epidemiology and End Results as sponsored by the National Cancer Institute. In light of ongoing rise

in melanoma incidence amongst the elderly and associated poor prognosis, additional work in this area is especially critical to our understanding and treatment of these patients.

REFERENCES

1. Morton DL, Wen DR, Wong JH, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg* 1992;127:392-9.

2. Morton DL, Thompson JF, Cochran AJ, et al. Sentinel-node biopsy or nodal observation in melanoma. *N Engl J Med* 2006;355:1307-17.

3. Balch CM, Soong SJ, Gershenwald JE, et al. Prognostic factors analysis of 17,600 melanoma patients: Validation of the american joint committee on cancer melanoma staging system. *J Clin Oncol* 2001;19:3622-34.

4. Statius Muller MG, van Leeuwen PA, de Lange-De Klerk ES, et al. The sentinel lymph node status is an important factor for predicting clinical outcome in patients with stage i or ii cutaneous melanoma. *Cancer* 2001;91:2401-8.

5. Austin PF, Cruse CW, Lyman G, et al. Age as a prognostic factor in the malignant melanoma population. *Ann Surg Oncol* 1994;1:487-94.

6. Chao C, Martin RC, 2nd, Ross MI, et al. Correlation between prognostic factors and increasing age in melanoma. *Ann Surg Oncol* 2004;11:259-64.

7. Sassen S, Shaw HM, Colman MH, et al. The complex relationships between sentinel node positivity, patient age, and primary tumor desmoplasia: Analysis of 2303 melanoma patients treated at a single center. *Ann Surg Oncol* 2008;15:630-7.

8. Sondak VK, Taylor JM, Sabel MS, et al. Mitotic rate and younger age are predictors of sentinel lymph node positivity: Lessons learned from the generation of a probabilistic model. *Ann Surg Oncol* 2004;11:247-58.

9. Fenske NA,Lober CW. Structural and functional changes of normal aging skin. *J Am Acad Dermatol* 1986;15:571-85.

10. Fenske NA,Lober CW. Skin changes of aging: Pathological implications. *Geriatrics* 1990;45:27-35.

11. Orlov RS, Borisova RP,Mandryko ES. [the contractile and electrical activity of the smooth muscles of the major lymph vessels]. *Fiziol Zh SSSR Im I M Sechenova* 1975;61:1045-53.

12. Gashev AA,Zawieja DC. Hydrodynamic regulation of lymphatic transport and the impact of aging. *Pathophysiology* 2010;

13. Gasheva OY, Knippa K, Nepiushchikh ZV, et al. Age-related alterations of active pumping mechanisms in rat thoracic duct. *Microcirculation* 2007;14:827-39.

14. Hadamitzky C, Spohr H, Debertin AS, et al. Age-dependent histoarchitectural changes in human lymph nodes: An underestimated process with clinical relevance? *J Anat* 2010;

15. Gervasoni JE, Jr., Taneja C, Chung MA, et al. Biologic and clinical significance of lymphadenectomy. *Surg Clin North Am* 2000;80:1631-73.

16. Conway WC, Faries MB, Nicholl MB, et al. Age-related lymphatic dysfunction in melanoma patients. *Ann Surg Oncol* 2009;16:1548-52.

17. American cancer society. What are the key statistics about melanoma? Http://www.Cancer.Org/docroot/cri/content/cri_2_4_1x_what_are_the_key_statistics_for_melanoma_50.Asp. Accessed june 25, 2010.

18. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 ajcc melanoma staging and classification. *J Clin Oncol* 2009;27:6199-206.

19. Morton DL, Thompson JF, Essner R, et al. Validation of the accuracy of intraoperative lymphatic mapping and sentinel lymphadenectomy for early-stage

melanoma: A multicenter trial. Multicenter selective lymphadenectomy trial group. *Ann Surg* 1999;230:453-63; discussion 63-5.

20. Morton DL, Cochran AJ, Thompson JF, et al. Sentinel node biopsy for early-stage melanoma: Accuracy and morbidity in mslt-i, an international multicenter trial. *Ann Surg* 2005;242:302-11; discussion 11-3.

21. Uren RF, Howman-Giles RB, Thompson JF, et al. Variability of cutaneous lymphatic flow rates in melanoma patients. *Melanoma Res* 1998;8:279-82.

22. Faries MB, Bedrosian I, Reynolds C, et al. Active macromolecule uptake by lymph node antigen-presenting cells: A novel mechanism in determining sentinel lymph node status. *Ann Surg Oncol* 2000;7:98-105.

23. Uren RF, Howman-Giles RB,Thompson JF. Demonstration of second-tier lymph nodes during preoperative lymphoscintigraphy for melanoma: Incidence varies with primary tumor site. *Ann Surg Oncol* 1998;5:517-21.

24. Bostick P, Essner R, Glass E, et al. Comparison of blue dye and probe-assisted intraoperative lymphatic mapping in melanoma to identify sentinel nodes in 100 lymphatic basins. *Arch Surg* 1999;134:43-9.

25. Carlson GW, Murray DR, Thourani V, et al. The definition of the sentinel lymph node in melanoma based on radioactive counts. *Ann Surg Oncol* 2002;9:929-33.

26. McMasters KM, Reintgen DS, Ross MI, et al. Sentinel lymph node biopsy for melanoma: How many radioactive nodes should be removed? *Ann Surg Oncol* 2001;8:192-7.

27. Essner R, Bostick PJ, Glass EC, et al. Standardized probe-directed sentinel node dissection in melanoma. *Surgery* 2000;127:26-31.

28. Cox CE, Dupont E, Whitehead GF, et al. Age and body mass index may increase the chance of failure in sentinel lymph node biopsy for women with breast cancer. *Breast J* 2002;8:88-91.

29. Bourgeois P. Scintigraphic investigations of the lymphatic system: The influence of injected volume and quantity of labeled colloidal tracer. *J Nucl Med* 2007;48:693-5.

30. Carlson GW, Murray DR, Lyles RH, et al. The amount of metastatic melanoma in a sentinel lymph node: Does it have prognostic significance? *Ann Surg Oncol* 2003;10:575-81.

31. Orlov RS, Borisova RP, Bubnova NA, et al. [the lymphatic vessels: Their tonus, motility and regulation]. *Fiziol Zh SSSR Im I M Sechenova* 1991;77:140-9.

32. Luscieti P, Hubschmid T, Cottier H, et al. Human lymph node morphology as a function of age and site. *J Clin Pathol* 1980;33:454-61.

33. Mortimer PS. Evaluation of lymphatic function: Abnormal lymph drainage in venous disease. *Int Angiol* 1995;14:32-5.

34. Weissleder H,Weissleder R. Lymphedema: Evaluation of qualitative and quantitative lymphoscintigraphy in 238 patients. *Radiology* 1988;167:729-35.

35. Rubin JR,Eberlin LB. The effect of inguinal lymphatic manipulation on regional lymph flow patterns. *J Vasc Surg* 1993;17:896-900; discussion 00-1.

36. Carlson GW, Page AJ, Cohen C, et al. Regional recurrence after negative sentinel lymph node biopsy for melanoma. *Ann Surg* 2008;248:378-86.

37. Scoggins CR, Martin RC, Ross MI, et al. Factors associated with false-negative sentinel lymph node biopsy in melanoma patients. *Ann Surg Oncol* 2010;17:709-17.

38. Pan WR, Suami H,Taylor GI. Lymphatic drainage of the superficial tissues of the head and neck: Anatomical study and clinical implications. *Plast Reconstr Surg* 2008;121:1614-24; discussion 25-6.

39. Rossi CR, De Salvo GL, Trifiro G, et al. The impact of lymphoscintigraphy technique on the outcome of sentinel node biopsy in 1,313 patients with cutaneous melanoma: An italian multicentric study (solism-imi). *J Nucl Med* 2006;47:234-41.

40. Strand SE,Persson BR. Quantitative lymphoscintigraphy i: Basic concepts for optimal uptake of radiocolloids in the parasternal lymph nodes of rabbits. *J Nucl Med* 1979;20:1038-46.

41. Pepper MS, Tille JC, Nisato R, et al. Lymphangiogenesis and tumor metastasis. *Cell Tissue Res* 2003;314:167-77.

TABLES

Table 1. Overall clinicopathologic characteristics of entire study population.

Variable	Total (n=1349)
Age (years)	52.1±15.1
Male gender	803 (59.5)
Primary sites	
Head & neck	214 (15.9)
Trunk	554 (41.0)
Upper limb	287 (21.3)
Lower limb	294 (21.8)
Breslow depth (mm)	2.43±2.07
Ulceration	312 (23.1)
Regression	232 (17.2)
Mitotic figures (per 1mm^2)	2.41±4.01
Histology	
Superficial spreading	677 (50.2)
Nodular	271 (20.1)
Unclassified	207 (15.3)
Lentigo maligna	96 (7.1)
Other	63 (4.7)
Acral lentiginous	35 (2.6)
Number of nodal basins	
1	1101 (81.6)
2	225 (16.7)
≥ 3	23 (1.7)
Number of removed SLN	2.51±1.81
Hottest node location	
Axilla	717 (53.2)
Inguinal	367 (27.2)
Cervical	239 (17.7)
In-transit	26 (1.9)

Table 2. Trends in clinicopathologic variables across age categories. All values expressed as mean±standard deviation or n (%) as appropriate.

	<30y (n=108)	**30-39y (n=184)**	**40-49y (n=292)**	**50-59y (n=305)**	**60-69y (n=278)**	**≥70y (n=182)**	**p[¥]**
Male gender	48 (44.4)	90 (48.9)	156 (53.4)	206 (67.5)	190 (68.3)	113 (62.1)	<0.001
Head & neck primary	19 (17.6)	16 (8.7)	35 (12.0)	42 (13.8)	60 (21.6)	42 (23.1)	<0.001
Breslow depth (mm)	2.20±1.57	1.84±1.49	2.29±1.96	2.38±2.27	2.73±2.13	2.42±2.07	<0.001
Ulceration	21 (19.4)	31 (16.8)	70 (24.0)	62 (20.3)	72 (25.9)	56 (30.8)	0.003
Radiocolloid dose (μCi)	346±246	331±247	359±321	309±213	337±293	353±404	0.43
Hottest node location							
Axilla	55 (50.9)	88 (47.8)	169 (57.9)	177 (58.0)	138 (49.6)	90 (49.5)	0.001
Inguinal	34 (31.5)	73 (39.7)	74 (25.3)	75 (24.6)	67 (24.1)	44 (24.2)	
Cervical	18 (16.7)	19 (10.3)	45 (15.4)	48 (15.7)	66 (23.7)	43 (23.6)	
In-transit	1 (0.9)	4 (2.2)	4 (1.4)	5 (1.6)	7 (2.5)	5 (2.7)	
Draining basins							
Single	93 (86.1)	148 (80.4)	229 (78.4)	251 (82.3)	222 (79.9)	158 (86.8)	0.17
Multiple	15 (13.9)	36 (19.6)	63 (21.6)	54 (17.7)	56 (20.1)	24 (13.2)	
Number of removed SLN	2.5±2.0	2.5±1.5	2.4±1.4	2.5±1.8	2.6±2.3	2.6±1.9	0.81

[¥]p-values obtained by one-way ANOVA, chi-square test for trend or Spearman's rank correlation as appropriate.

Table 3. Results from univariate and multivariate analyses of relationships between clinicopathologic variables and hottest radioactive count rates. Final linear regression model included age, ulceration, days between injection and SLN biopsy, radiocolloid dose, number of SLN, and location of SLN basin. All values expressed as mean±standard deviation or n (%) as appropriate.

Variables	**Mean Hottest Count Rates (per 10 seconds)**	**p-value[ȷ]**	**Estimated Change in Hottest Count Rates (per 10 seconds) by multiple linear regression**	**p-value**
Age (years, continuous)	-	<0.001	-109.9	<0.001
Gender				
Female	8259	0.59	-	-
Male	7947			
Primary Site				
Head & neck	6724	0.04[ε]	-[ε]	-[ε]
Non-head &neck	8328			
Breslow depth (mm, continuous)	-	0.50	-	-
Ulceration				
Present	9154	0.07	+1596.7	0.02
Absent	7749		reference	
Days between injection and SLN biopsy				
One day	2831	0.19	-7094.8	0.07
Same day	8101		reference	
Radiocolloid dose (μCi, continuous)	-	0.04	+2.3	0.02
Number SLN retrieved (continuous)	-	<0.001	+622.9	<0.001
Number of draining basins				
Single basin	8072	0.99	-	-
Multiple basins	8080			
Hottest SLN pathology				
Positive	8886	0.34	-	-
Negative	7946			
Hottest SLN basin				
Axilla	8188	0.03	-883.6	0.21
Cervical	6498		-2265.1	0.01
In-transit	6484		-2942.2	0.18
Inguinal	8990		reference	
Overall SLN pathology				
Positive	8594	0.46	-	-
Negative	7960			

[ȷ]Univariate p-values obtained from independent sample t-tests for binary variables, one-way ANOVA for three or more categorical variables, and Pearson's correlation coefficients for continuous variables.
[ε]Primary site is clinically closely related to SLN basin, and was dropped from the final linear regression model on multivariate analysis.

Table 4. Patient and tumor characteristics in patients with negative and positive SLN. All values expressed as mean±standard deviation or n (%) as appropriate.

Variable	**SLN negative (n=1107)**	**SLN positive (n=242)**	**p-value**
Age (years)	52.6±15.0	50.2±15.6	0.026
Male gender	638 (57.6)	165 (68.2)	0.002
Primary sites			
Head & neck	178 (16.1)	36 (14.9)	0.014
Trunk	440 (39.7)	114 (47.1)	
Upper limb	253 (22.9)	34 (14.0)	
Lower limb	236 (21.3)	58 (24.0)	
Breslow depth (mm)	2.18±1.83	3.51±2.68	<0.001
Ulceration	217 (19.6)	95 (39.3)	<0.001
Regression	200 (18.1)	32 (13.2)	0.07
Mitotic figures (per 1mm^2)	2.12±3.71	3.74±4.97	<0.001
Histology			
Superficial spreading	552 (50.0)	125 (51.7)	0.003
Nodular	211 (19.1)	60 (24.8)	
Unclassified	181 (16.3)	26 (10.7)	
Lentigo maligna	88 (7.9)	8 (3.3)	
Other	51 (4.6)	12 (5.0)	
Acral lentiginous	24 (2.1)	11 (4.5)	
Number of nodal basins			
1	921 (83.2)	180 (74.4)	0.004
2	167 (15.1)	58 (24.0)	
≥ 3	19 (1.7)	4 (1.6)	
Number of removed SLN	2.47±1.81	2.71±1.79	0.059
Hottest node location			
Axilla	599 (54.1)	118 (48.8)	0.008
Inguinal	297 (26.8)	70 (28.9)	
Cervical	196 (17.7)	43 (17.8)	
In-transit	15 (1.4)	11 (4.5)	

Table 5. Binary logistic regression model for SLN disease.

Covariates	Odds Ratio	95% Confidence Interval	P-value
Age (years)	0.98	0.97-0.99	<0.001
Male gender	1.76	1.27-2.45	0.001
Head & neck primary site[δ]	0.57	0.34-0.57	0.027
Breslow depth (mm)	1.28	1.20-1.37	<0.001
Ulceration	2.10	1.52-2.90	<0.001

[δ]Reference group lower extremity.

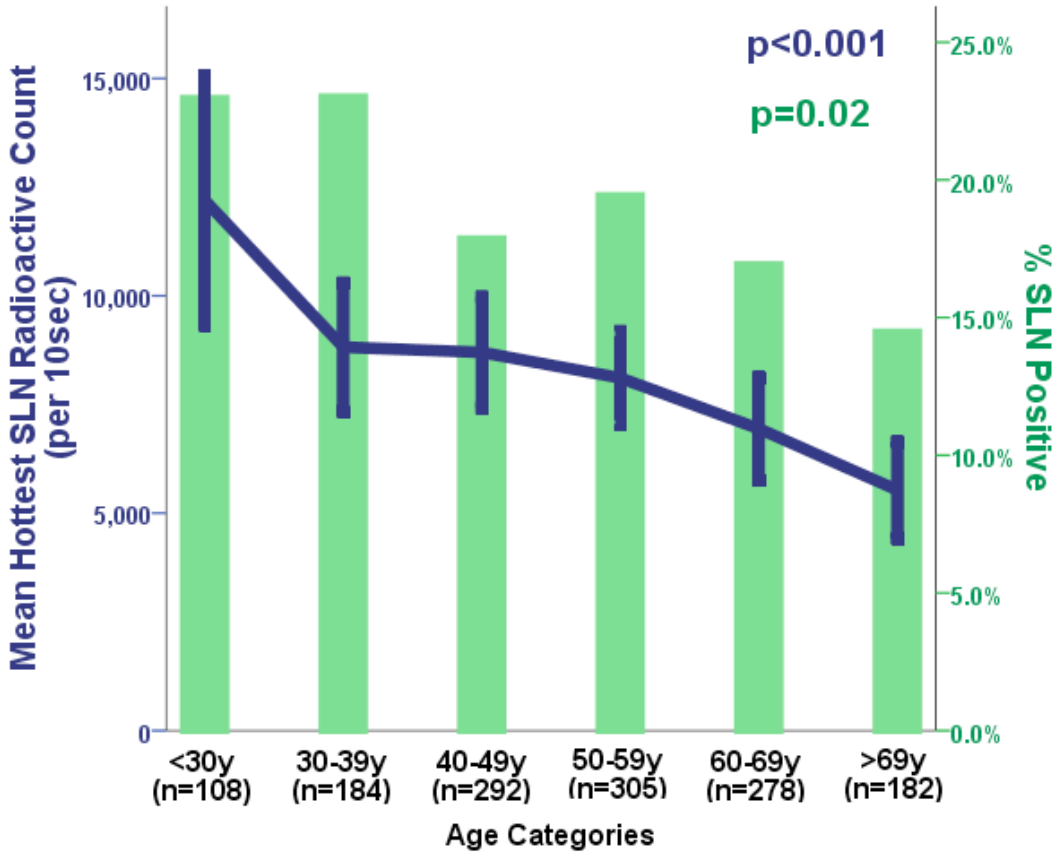

Figure 1. With increasing age, mean hottest ex-vivo radioactivity count rates (by 6 age categories in ascending order, 12200, 8820, 8700, 8110, 6944, and 5531 per 10 seconds, $p<0.001$) and SLN-positive rates (22.2, 22.3, 17.1, 18.7, 16.2, and 13.7%, $p=0.02$) both decline. Error bars represent 95% confidence intervals around means.