Impact of Biopsy Technique on Clinically Important Outcomes for Cutaneous Melanoma: A Systematic Review and Meta-analysis

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Abstract

We performed a systematic review and meta-analysis to examine the relationship between the type of biopsy technique employed in the diagnosis of cutaneous melanoma and 4 clinically important outcomes: melanoma-specific mortality, all-cause mortality, Breslow tumor depth, or melanoma recurrence. Our database was obtained by searching PubMed, Ovid MEDLINE, EMBASE, the Cochrane Database of Systematic Reviews, and the Cochrane Library from inception until December 6, 2019. Studies were identified that compared biopsy techniques used to diagnose cutaneous melanoma with any of our study outcomes. We included 7 observational studies for our meta-analysis after screening 3231 titles and abstracts. Pooled data identified a significantly higher all-cause mortality in the punch biopsy group (risk ratio [RR], 1.520; \( P = .02 \)). A higher, but nonsignificant, rate of melanoma-specific mortality (RR, 1.96; \( P = .22 \)) and melanoma recurrence (RR, 1.20; \( P = .186 \)) was also found for the punch biopsy group. Breslow tumor thickness was not significantly lower for punch incision (standardized mean difference, \(-0.42; \ P = .27 \)). We found limited evidence for differences in clinically important outcomes across the spectrum of the most common methods employed in clinical practice for the initial diagnosis of cutaneous melanoma. A small, but significant, increase (\( P = .02 \)) in all-cause mortality with punch biopsies was not seen for the other outcomes and was most likely due to small sample sizes and demographic differences in the included studies and unlikely represents a clinically important outcome. Our findings support the use of existing clinical practice guidelines for evaluating pigmented lesions suspicious for cutaneous melanoma.

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Malignant melanoma continues to have an increasing incidence worldwide.\(^1\) Despite advances in the diagnosis and treatment of melanoma in the United States, a sustained decline in mortality has not been achieved.\(^2\) Cutaneous melanoma (CM) is diagnosed primarily by a visual skin examination and a subsequent skin biopsy for histologic confirmation and initial staging. In most countries, the diagnosis is typically made by a primary care physician or dermatologist. Guidelines from Australia, Europe, the United Kingdom, and the United States all recommend a full-thickness excision (including the entire epidermis and dermis and to the level of the subcutaneous fat) as the diagnostic procedure of choice for skin lesions that are clinically suggestive of melanoma.\(^3-5\) Evidence supports the use of full-thickness excisional biopsies for the attainment of the best histologic and Breslow tumor depth accuracy.\(^6-8\) The practice of full-thickness excision with 1- to 3-mm lateral margins around the pigmented lesion allows for the most detailed pathologic information, which is vital for staging and treatment decisions.\(^6\) Guidelines from the US American Academy of Dermatology outline 3 methods to accomplish a diagnostic excisional biopsy: (1) elliptical excision, (2) punch excision...
around the clinical lesion, or (3) deep shave (saucerization) extending to the deep reticular dermis. It is extremely important for all of these techniques to be employed with lateral margins of 1 to 3 mm and full thickness in depth.

In current practice, physicians utilize an array of biopsy techniques because of physician or patient preferences, challenging anatomic locations, and lesion sizes. The most commonly utilized biopsy techniques for the diagnosis of CM include (1) complete excisional biopsy using a scalpel, (2) incisional biopsy using a scalpel, (3) excisional punch biopsy with the entirety of the lesion confined within the surface area of the punch instrument, (4) incisional punch biopsy with only a portion of the lesion being removed, and (5) a deep shave biopsy with the use of either a scalpel or razor blade. The deep shave or saucerization (scooping with a razor blade or scalpel) is the most common technique employed by dermatologists because of its ease of use and efficiency. Concern has been raised over the use of superficial shave biopsies (not to the level of the reticular dermis) and superficial punch biopsies (less than full thickness in depth), which may result in histopathologic misdiagnosis, tumor transection at its deep margins, and even increased mortality. Incisional punch biopsies carry a risk of sampling error because not all of the lesion is obtained for histologic analysis. Therefore, incisional punch biopsies can lead to a missed diagnosis of a melanoma or an underestimation of true Breslow depth. Thus all current guidelines currently warn against partial incisions for the evaluation of pigmented lesions suspicious for CM.

Given the wide array of clinical presentations and physician practice preferences, it would be expected to see variability in the diagnostic evaluation of pigmented skin lesions suspicious for melanoma. We aimed to examine and expand on the evidence base across the spectrum of biopsy types performed to diagnose CM.

Methods
We reported this systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement. After writing our study question (Supplemental Material, available online at http://www.mcpqojournal.org), we searched the English language literature using medical subject heading terms and text words for common indexing practices from inception of the chosen databases until December 6, 2019. PubMed, Ovid MEDLINE, EMBASE, Cochrane Database of Systematic Reviews, and the Cochrane Library were searched by 2 investigators (R.A.S., F.F.) using the following terms: melanoma, screening, biopsy type, biopsy technique, diagnostic techniques, clinical outcomes, mortality, all-cause mortality, melanoma-specific mortality, recurrence, Breslow thickness, punch biopsy, shave biopsy, deep shave biopsy, saucerization, incisional biopsy, and excisional biopsy. Our search was augmented by author and reference tracking to identify additional studies.

Article Highlights
- To our knowledge, this study is the first systematic review and meta-analysis to search for a relationship between diagnostic biopsy techniques and clinically important outcomes in cutaneous melanoma.
- Existing clinical practice guidelines recommend a full-thickness excisional biopsy as the preferred procedure to diagnose a suspected melanoma. Despite these recommendations, variability in clinical practice exists.
- We have identified a small but statistically significant increase ($P = .02$) in all-cause mortality for punch biopsies used to diagnose cutaneous melanoma. This difference was not seen for any other clinically important outcomes and may be explained by differences in patient demographic characteristics in our included studies.
- Our findings give further support to the current clinical practice guidelines for the evaluation of pigmented lesions suspected for the diagnosis of cutaneous melanoma.
- These findings elucidate the need to further expand the database in an effort to guide future clinical practice in the diagnostic evaluation of pigmented lesions suspicious for cutaneous melanoma.
After reviewing all titles and abstracts, we included studies based on full-text reviews (Figure 1). Inclusion criteria were studies of cohorts or case series of patients diagnosed with CM having at least 2 comparison groups and 1 of 3 biopsy types: (1) elliptical excisional biopsy employing a scalpel, (2) punch biopsy, or (3) shave biopsy. The final inclusion criteria were the presence of data collection regarding melanoma-specific mortality, all-cause mortality, Breslow tumor thickness, or melanoma recurrence for each biopsy type. We preferred prospective studies but allowed retrospective studies that included the entire patient population of a cohort, case control, or case series. Exclusion criteria included studies that did not record any of our chosen outcome measures, had greater than 20% of the enrolled patients lost to follow-up, were case reports, were abstract-only publications, and were in a language other than English. We also excluded trials that selected only a portion of a defined cohort, case series, or case control to be studied (Supplemental Material, available online at http://www.mcpiqojournal.org).

Two investigators (R.A.S., F.F.) independently reviewed each retrieved article, and all data were extracted from full-text articles, including tables and figures. Disagreements were addressed by consensus and by a third reviewer (M.N.). We extracted the following characteristics from each study: primary author, time period of the study, year of publication, patient baseline characteristics, and outcomes of interest. Interobserver agreement...
for study selection had a $\kappa$ statistic of 0.645. All 3 investigators independently assessed the quality and risk of bias of all 7 included studies using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies available from the National Institutes of Health.\textsuperscript{16}

A meta-analysis was performed for 4 outcomes: melanoma-specific mortality, all-cause mortality, Breslow tumor thickness, and melanoma recurrence. Data were insufficient to include shave biopsies; therefore, the comparison groups were limited to punch biopsy and excisional biopsy. The mortality and recurrence outcomes are binary, and the pooled effect size reported is the risk ratio (RR). For Breslow thickness, the standardized mean difference (SMD) is reported. The pooled RR is based on 3 studies for all-cause melanoma and 2 studies for melanoma-specific mortality. The pooled RR for recurrence is based on 3 studies. Only 2 studies reported sufficient statistics (mean and SD) to compute the effect size for Breslow thickness. A random-effects model was used given the universality of the database search and heterogeneity of the patient populations of our included studies. Heterogeneity among studies was assessed using the $\chi^2$ test and the $I^2$ statistic. $P$ values are 2-sided with statistical significance defined as $P<.05$. The statistical analysis was performed in R version 3.6.0 using the meta package.\textsuperscript{17}

### RESULTS

We identified 3231 studies in our initial selection phase for review. After initial exclusion during abstract and title review, we identified 212 relevant articles for full-text review (Figure 1). We initially included 8 studies based on our predetermined criteria.\textsuperscript{18-25} After a disagreement about Bong et al,\textsuperscript{19} this study was excluded by consensus because only a portion of a patient population from a national cancer registry were selected for this retrospective case series. Therefore, 7 articles were selected for our meta-analysis (Table 1).\textsuperscript{18,20-25} These 7 included trials enrolled a total of 4374 patients who underwent skin biopsies for the diagnosis of CM. Of the 4374 patients, melanoma diagnosis was made by excisional biopsy in 2934, by shave biopsy in 707, and by punch biopsy in 660. Study design characteristics included 3 prospective cohorts, 2 case series, 1 retrospective case control, and 1 retrospective case series.

| Reference, year | Type of study | No. of participants | Mean age (y) | Males (%) | Ethnicity | Duration (y) | Types of biopsies | Mean follow-up (y) |
|----------------|--------------|---------------------|-------------|-----------|-----------|-------------|------------------|------------------|
| Namin & Zitsch,\textsuperscript{18} 2018 | Retrospective case series | 170 | 63 | 75 | NA | 5 | Shave, punch, excisional, incisional | 3.1 |
| Mir et al,\textsuperscript{20} 2013 | Retrospective case control | 479 | 68 | 97 | NA | 8 | Shave, punch, excisional | NA |
| Molenkamp et al,\textsuperscript{21} 2007 | Prospective cohort | 440 | 50 | 47 | NA | 11 | Punch and excisional | 5.1 |
| Martin et al,\textsuperscript{22} 2005 | Prospective cohort | 1782 | 50 | 57 | NA | 2.5 | Shave, punch, and excisional | 2.5 |
| Austin et al,\textsuperscript{23} 1996 | Case series | 159 | 49 | 74 | 99% White | 8 | Excisional and punch | 3.2 |
| Griffiths & Briggs,\textsuperscript{24} 1985 | Case series | 258 | NA | NA | NA | 6 | Punch, excisional with narrow margin, excisional with wide margin | 10.0 |
| Lees & Briggs,\textsuperscript{25} 1991 | Prospective cohort | 1086 | 53 | 28 | NA | 17 | Punch, narrow excision, wide excision | 5.0 |

NA = not applicable.
There were 2 trials that included only patients with melanoma of the head and neck.\(^\text{18,23}\) The mean age of patients ranged from 49 to 68 years. Males comprised 55.4% of the sample size (2281 of 4116 patients), with one study of 258 patients not reporting age, sex, or ethnicity.\(^\text{17}\)

The result of pooling the all-cause mortality studies also revealed a significantly higher rate of death among the punch biopsy group;
the RR using the random-effects model was 1.52 (95% CI, 1.08-2.13; P=.02) (Figure 2A). Publication bias is difficult to assess with only 3 data points; however, 2 of the 3 studies cluster to the right of the combined effect size, suggesting that the results are biased by studies with higher RRs (Figure 3A).

For studies that tracked melanoma-specific mortality, the pooling identified a higher, but
| Variable                                                                 | Namin & Zitsch, 2018 | Mir et al., 2013 | Molenkamp et al., 2007 | Martin et al., 2005 | Austin et al., 1996 | Griffiths & Briggs, 1985 | Lees & Briggs, 1991 |
|-------------------------------------------------------------------------|----------------------|------------------|------------------------|---------------------|----------------------|------------------------|---------------------|
| Quality assessment                                                      | Yes                  | Yes              | Yes                    | Yes                 | Yes                  | Yes                    | Yes                 |
| Were all the participants selected or recruited from the same or similar populations? | Yes                  | Yes              | Yes                    | Yes                 | No                   | Yes                    | Yes                 |
| Was the time frame sufficient that one could reasonably expect to see an association between exposure and outcome if it existed? | Yes                  | Yes              | Yes                    | No                  | No                   | Yes                    | Yes                 |
| Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants? | Yes                  | Yes              | Yes                    | Yes                 | Yes                  | No                     | Yes                 |
| Were the outcome assessors blinded to the exposure status of participants? | No                   | No               | Yes                    | Yes                 | Yes                  | Yes                    | Yes                 |
| Was loss to follow-up after baseline 20% or less?                       | Yes                  | Yes              | Yes                    | Yes                 | Yes                  | Yes                    | Yes                 |
| Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)? | Yes                  | Yes              | Yes                    | Yes                 | Yes                  | No                     | Yes                 |

**Risk of bias**

| Risk of bias                                                                 | Low risk | High risk | High risk | High risk | Low risk | High risk | High risk |
|-------------------------------------------------------------------------------|----------|-----------|-----------|-----------|----------|-----------|-----------|
| Selection bias—comparable cohorts or case series                             | Low risk | High risk | High risk | High risk | Low risk | High risk | High risk |
| Information bias—misclassification                                            | Low risk | Low risk  | Low risk  | Low risk  | Low risk | Low risk  | Low risk  |
| Measurement bias—outcome measures errors or lack of blinding                 | Moderate risk | Moderate risk | Low risk  | Low risk  | Low risk | Moderate risk | Low risk  |
| Cofounders—variables adjusted for impact                                      | Low risk | Low risk  | Low risk  | Low risk  | Low risk | Low risk  | Low risk  |
nonsignificant, rate of death among those in the punch biopsy group. The pooled RR using the random-effects model was 1.96 (95 % CI, 0.67-5.71; P=.22) (Figure 2B). Results of “leave-one-out” influence analyses revealed that the significance of the pooled estimate does not change after omitting any of the studies.

Pooling the 2 studies that examine Breslow thickness revealed that values in the punch biopsy group were lower, with an SMD of −0.42; (P=.27); however, this difference is not significant (Figure 2D). Because there were only 2 studies considered for this outcome, influence analysis was not conducted.

Finally, the pooled RR for recurrence was not significant (RR, 1.20; P=.186; Figure 2C). The funnel plot does not show evidence of publication bias affecting the result, although the number of studies is again very small (Figure 3). Results of “leave-one-out” influence analyses revealed that the significance of the pooled estimate does not change after omitting any of the studies.

Influence analysis, publication bias assessment, and quality assessment were performed on all included studies. As noted previously, significance in the outcomes did not change after conducting influence analyses on the studies considered.

Three investigators (R.A.S., F.F., M.N.) reviewed all included studies and independently evaluated each for risk of bias and quality. Risk of bias was assessed using the Cochrane collaborative tool for assessing risk of bias26 (Table 2). The main sources of bias identified in our 7 studies were selection bias and measurement bias. Five of our included cohort studies and case series did not have equal numbers of patients undergoing each biopsy type, which is an example of selection bias. The outcomes assessments were not blinded to the investigators in 3 of our included studies. Funnel plots for publication bias are included in Figure 3.

Quality assessment was performed using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies available from the National Institutes of Health16 (Table 2). The quality level of evidence from our included studies was level III according to the Oxford Centre for Evidence-Based Medicine rating system.27 Despite these shortcomings, the overall quality was assessed to be fair, and the risk of bias was moderate.

**DISCUSSION**

Identification of possible differences in clinically important outcomes based on the type of biopsy performed in the diagnosis of CM could have important implications for clinical practice. The 4 clinically important outcomes we studied were all-cause mortality, melanoma-specific mortality, Breslow tumor thickness, and melanoma recurrence. The mortality data are obviously the most important outcome. Recurrence of melanoma is a strong predictor of mortality in CM and adds to the public health burden of melanoma, which has become one of the most costly solid malignancies in the United States.19,21,28,29 Breslow thickness has a very strong predictive correlation with 2 of our clinically important outcomes: mortality and recurrence.30-32 To our knowledge, our study is the only meta-analysis to determine whether differences existed in relation to our chosen clinically important outcomes for the 3 biopsy types.

We performed our meta-analysis on 7 studies. There was not enough data comparing our outcomes measures for shave biopsy; therefore, our meta-analysis was limited to excisional biopsies (with a scalpel) and punch biopsies. Excisional biopsies were performed in all 7 studies according to published guidelines and were performed to obtain 1- to 3-mm lateral margins and a full-thickness depth. Punch biopsies were also performed in all 7 included studies but were considered to remove a partial portion of the melanoma in nearly all cases. Only the study by Namin and Zitsch18 described a punch biopsy that was intended to excise the entire skin lesion.

Our most interesting finding was a higher rate of all-cause mortality showing a statistically significant rate of death in the punch biopsy group (RR, 1.520; P=.02). The result of pooling the studies that track melanoma-specific mortality found a higher but nonsignificant rate of death among those in the punch incision group (RR, 1.96; P=.15). An explanation for the increase in all-cause mortality in the patients having their melanoma diagnosed with a punch incision is not
BIOPSY FOR MELANOMA OUTCOMES

apparent by any obvious technical differences in the method or performing this procedure. We did find that 2 of the studies that tracked all-cause mortality included older patients in the punch incision group. The punch incision group in the study by Namin and Zitsch had a mean age that was 7 years older than that of the excisional biopsy group, and the study by Austin et al had a 19-year higher median age in the punch incision group. Older age could explain this higher all-cause mortality. Patient selection may also influence biopsy type, and patient demographic characteristics and comorbidities were not reported in any of our included studies. Inconsistencies in the accuracy of reporting the cause of death have also been noted in cancer screening studies. Publication bias may also account for these differences because one large outlier may have raised the pooled estimate higher (Figure 3). Therefore, the finding of an inconsistency between all-cause mortality and melanoma-specific mortality in our study is most likely due to the previously mentioned factors and is not clinically important enough to have potential implications for clinical practice. We find no reason to change the clinical practice of employing punch incision for diagnosing CM as outlined in the US guidelines.

No significant differences were seen with regard to melanoma recurrence for the excisional biopsy and punch incision groups (RR, 1.20; P=.186). Finally, Breslow thickness was nonsignificantly lower in the punch incision group (SMD, -0.42; P=.27). Although the data are from only 2 studies, a large sample size was represented. Lower Breslow thickness in punch incisions may be accounted for by the possibility of these procedures resulting in partial-thickness biopsies, which may underestimate the Breslow depth. Because data on the thickness of these punch incisions were not included, we could not make any conclusions regarding Breslow thickness for different types of biopsy.

Given the clinical diversity of CM, it is not surprising to identify a lack of uniformity in biopsy types performed to diagnose CM. Results from our meta-analysis revealed a variety of biopsy types being performed to diagnose CM. Data from our 7 studies included 4301 biopsies with a confirmed diagnosis of CM. Excisional biopsy was performed in 68.2% (2934 of 4301) of the cases, while shave biopsy and punch incision were performed in 16.4% (707 of 4301) and 15.3% (660 of 4301) of the cases, respectively. The US guidelines from the American Academy of Dermatology recommend that a full-thickness excision be performed on pigmented lesions for which the diagnosis of CM is suspected. Three options are suggested for performing the full-thickness excision: a full-thickness elliptical excision with a scalpel, a deep shave or saucerization, or a full-thickness punch biopsy with safety margins around the lesion. These options are not specifically outlined in the guidelines from Australia, Europe, and the United Kingdom. Findings from our study reflect these different recommendations. Four of our studies were done in the United States (2550 diagnosed CM cases, 52% excision, 28% shave, and 20% punch). Three studies were done in the United Kingdom (2040 CM cases, 83% excision and 17% punch) and one in Europe (440 CM cases, 88% excision and 12% punch). Shave excisions were used to diagnose CM only in the United States, and punch biopsies were performed slightly less in the United Kingdom and Europe. The findings from our systematic review and meta-analysis support the recommendations of all of the established guidelines discussed previously.

We believe that our meta-analysis, although limited by the small number of studies, provides some important insights to suggest that more studies are needed to ensure the safety of current clinical practice guidelines. Deep shave or saucerization was employed as a diagnostic approach in 28% of CM cases from the 4 US studies included in our meta-analysis; however, there were not enough data available from our systematic review to analyze outcome measures for this procedure in our meta-analysis. Further studies on deep shave biopsies to diagnose CM may assist in identifying possible differences in clinically important outcomes. The concern for partial incisional biopsies adversely affecting patient outcomes by transferring melanoma cells into cutaneous lymphatics or blood vessels has not been studied extensively. Three prior studies comparing incisional and excisional biopsies to diagnose melanoma have not reported differences in...
sentinel lymph node positivity, melanoma metastasis, or disease recurrence. Further study in all of these areas should be helpful in guiding clinical practice.

The most notable limitation of our meta-analysis was the small number of quality studies found to answer our question and the lack of statistically significant data for all of the outcomes. Our quality assessment revealed moderate-quality studies, primarily due to our included trials being observational, case control, or cohort studies (Table 2). The risk of bias was also moderate, especially for selection bias and measurement bias (Table 2). Using only studies published in the English language may have introduced study selection bias and limited our ability to find statistically significant outcomes. Another source of potential bias when comparing studies on biopsy procedures is the potential for heterogeneity of surgical techniques that can make comparisons difficult. Punch biopsies in our included studies were most likely all partial incisional biopsies and not in accordance with the US guidelines. Only one of the studies indicated that the punches could remove either a portion or the entirety of the lesion. Data were not published but were obtained from the authors of this study. Of the 47 patients who underwent a fully excised punch biopsy, 5 of 33 died during a mean 3.1-year follow-up, and 1 of 14 who underwent partial incisions using the punch instrument died within the same time frame. The mortality difference was not explained by the Breslow thickness, which were not statistically different. Future studies on higher numbers of patients diagnosed by full-thickness punch biopsies with 1- to 3-mm lateral margins may shed more light on the efficacy or safety of this procedure.

Our meta-analysis found limited evidence for differences in clinically important outcomes across the spectrum of the most common methods employed in clinical practice for the initial diagnosis of CM. The only statistically significant difference we identified was a small increase in all-cause mortality when punch biopsy was performed to diagnose melanoma (P=.02) without any differences in melanoma-specific mortality, melanoma recurrence, or Breslow thickness. The small difference in all-cause mortality was difficult to explain and does not likely have any clinical importance or impact on practice patterns. Our data do support the existing guidelines that provide recommendations for the diagnostic evaluation of pigmented skin lesions suspicious for CM.

**SUPPLEMENTAL ONLINE MATERIAL**

Supplemental material can be found online at http://www.mcpiqojournal.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

**Abbreviations and Acronyms:** CM = cutaneous melanoma; RR = risk ratio

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