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ORIGINAL ARTICLE – MELANOMA

Correlation Between Surgical and Histologic Margins in Melanoma Wide Excision Specimens

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ABSTRACT

Introduction. Wide surgical excision is the standard treatment for localized primary cutaneous melanomas, with a narrow histologic margin associated with an increased risk of local recurrence. The correlation between surgical and histologic margins is poorly documented in the literature.

Methods. An audit was performed to (1) document the shrinkage of formalin-fixed specimens, and (2) use a precisely measured surgical margin in vivo to predict the histologic margin. For patients presenting for wide excision of melanomas and other malignant skin tumors, measured surgical margin, in vivo and ex vivo specimen width, and histologic margins after formalin fixation were recorded. The effects of clinicopathologic characteristics, including age, sex, body mass index (BMI), tumor type, anatomic site, and presence of visible tumor in predicting specimen shrinkage and histologic margin were assessed.

Results. In total, 252 specimens were evaluated. When compared with measured width in vivo, the formalin-fixed specimens showed a mean shrinkage of 14% (R^2 = 0.98), regardless of patient age, sex, BMI, or site of the lesion. The measured surgical margin was not a strong predictor of the histologic margin, with a high degree of variability (R^2 = 0.55) not explained by patient factors, tumor subtype, or presence of visible tumor at the time of excision ($p > 0.05$).

Conclusions. A consistent 14% shrinkage rate of wide excision specimens was found across all patients and excision sites, and we propose a clinically useful 15% correction factor that will account for fixation and shrinkage of cutaneous excision specimens. Excision margins measured by the surgeon were a poor predictor of the histologic margins.

Complete surgical excision is a fundamental component of standard care for clinically localized cutaneous melanoma. The importance of adequate radial margins of excision was first suggested in 1885;[1] however, the matter was a topic of ongoing debate, prompting large, prospective, randomized trials seeking to define the surgical margins required to minimize local recurrence risk and avoid possible adverse effects on survival outcome.[2–6] Based on these data, national and international excision margin guidelines have been developed;[7, 8] however, these recommendations are based only on measured surgical margins, without any consideration of optimal histologic margins.

Previous studies from Melanoma Institute Australia (MIA) found an increased risk of local recurrence with histologic margins < 8 mm in T1 (< 1 mm) and T2 (1–2 mm) melanomas.[9–11] Another large MIA study found that histologic margins ≥ 16 mm were associated with better local control in patients with melanomas > 4 mm

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thick.[12] While these studies provided clues to the impact of excision margins on local recurrence in melanoma, none investigated the correlation between the actual surgical margins and the histologic margins. Rather, the surgical margin was estimated, based on previous studies that reported 20% tissue shrinkage after excision and fixation.[13,14]

Since there have been very few studies assessing the histological adequacy of surgical margins, we sought to carefully examine the degree of shrinkage in formalin-fixed wide excision specimens, determine the actual correlation between precisely measured surgical and histologic margins, and identify any patient or tumor characteristics that might affect these relationships.

METHODS

With institutional Ethics Committee approval, consecutive patients with biopsy-proven primary melanomas or non-melanoma skin cancers treated at MIA between March and July 2017 were prospectively included. Surgical wide excision margins were determined by the surgeon based on clinical assessment of each patient. During preoperative planning, the surgeon precisely measured and marked the planned excision margin ('surgical margin') from the edge of the biopsy scar or residual tumor. The width (shortest dimension) of the specimen prior to excision was also measured ('in vivo width') (Fig. 1a).

The lesions were excised by cutting along the outside of the marked pen line. Specimens were immediately placed in 10% neutral buffered formalin. After fixation, the specimen width was measured prior to sectioning ('ex vivo width') (Fig. 1b). The histologic margins for specimens with residual tumor were evaluated according to routine practice by pathologists at the Royal Prince Alfred Hospital. After inking the excision margins, the specimen was sliced into sequential 3-mm transverse sections, and each embedded in a paraffin block. Sections 5-μm-thick were cut from the formalin-fixed, paraffin-embedded tissue, examined microscopically, and the clearance of tumor, including melanoma in situ, from the margin was measured. Where no residual tumor remained, the histologic margins were measured from the edge of the excision biopsy scar ('histologic margin') (Fig. 1c).

Outcomes measured were (1) the degree of shrinkage of the formalin-fixed excision specimen compared with the measured specimen width in vivo, and (2) the correlation between surgically and histologically measured margins. Clinical and pathologic features were examined for their impact on specimen shrinkage and margin discrepancy.

Prior to data collection, a power analysis was performed to determine the number of specimens needed. Based on previous studies suggesting variable shrinkage in cutaneous specimens from excision to tissue fixation ranging from 11 to 20%,[13,15], we calculated that sample sizes of 100, 132, and 200 specimens would achieve $> 90\%$ power to detect a change in slope from 1.0 under the null hypothesis to 0.80, 0.85, and 0.90, respectively, under the alternative hypothesis. A two-sided significance level of 0.05 was assumed.

The clinicopathologic characteristics were summarized using mean [± standard deviation (SD)] or median (range) for continuous variables, and frequency (%) for categorical variables. The degree of shrinkage was determined using univariable linear regression for both (1) ex vivo and in vivo width, and (2) histologic and surgical margin. Additionally, subgroup analyses were performed using patient characteristics to determine whether the degree of shrinkage was associated with any of the following factors: sex (male vs. female), age (stratified by quartile, ≤ 55 years, 56–67 years, 68–75 years, > 75 years), body mass index [BMI; < 25 (underweight/normal) vs. ≥ 25 (overweight/obese)], or anatomic site of the lesion (head/face/scalp vs. neck vs. extremity vs. elbow/knee vs. torso). In addition to the clinical characteristics, pathologic features (tumor subtype defined as either melanoma in situ/atypical pigmented lesion (APL), invasive melanoma, or non-melanoma skin cancer) and the presence/absence of residual tumor were analyzed for possible impact on the correlation between surgical and histologic margins.

RESULTS

In total, 252 specimens from 240 patients were excised and evaluated. The clinicopathologic characteristics are detailed in Table 1. Just over half (54%) of the excised lesions were invasive melanomas, with a median thickness of 0.9 mm (range 0.15–8.8). The anatomic location of the specimens was evenly distributed across the trunk, extremity, and head/neck. Just over one-third of specimens (37.3%) contained clinically visible residual tumor, while the remainder contained scar only.

The in vivo width measurements ranged from 10 to 71 mm [mean 27.3 mm (SD 10.8 mm)], and measured ex vivo widths of formalin-fixed specimens ranged from 8 to 65 mm [mean 23.4 mm (SD 10.2 mm)]. The mean surgical margin was 9.8 mm (SD 4.2, range 2–20), and the mean histologic margin was 6.3 mm (SD 3.6, range 0–19).

When compared with the measured in vivo width, formalin-fixed specimens showed a mean shrinkage of 14% [95% confidence interval (CI) 12–15%]. The coefficient of determination was almost perfect ($R^2 = 0.98$), which means that 98% of the variance in the ex vivo width was

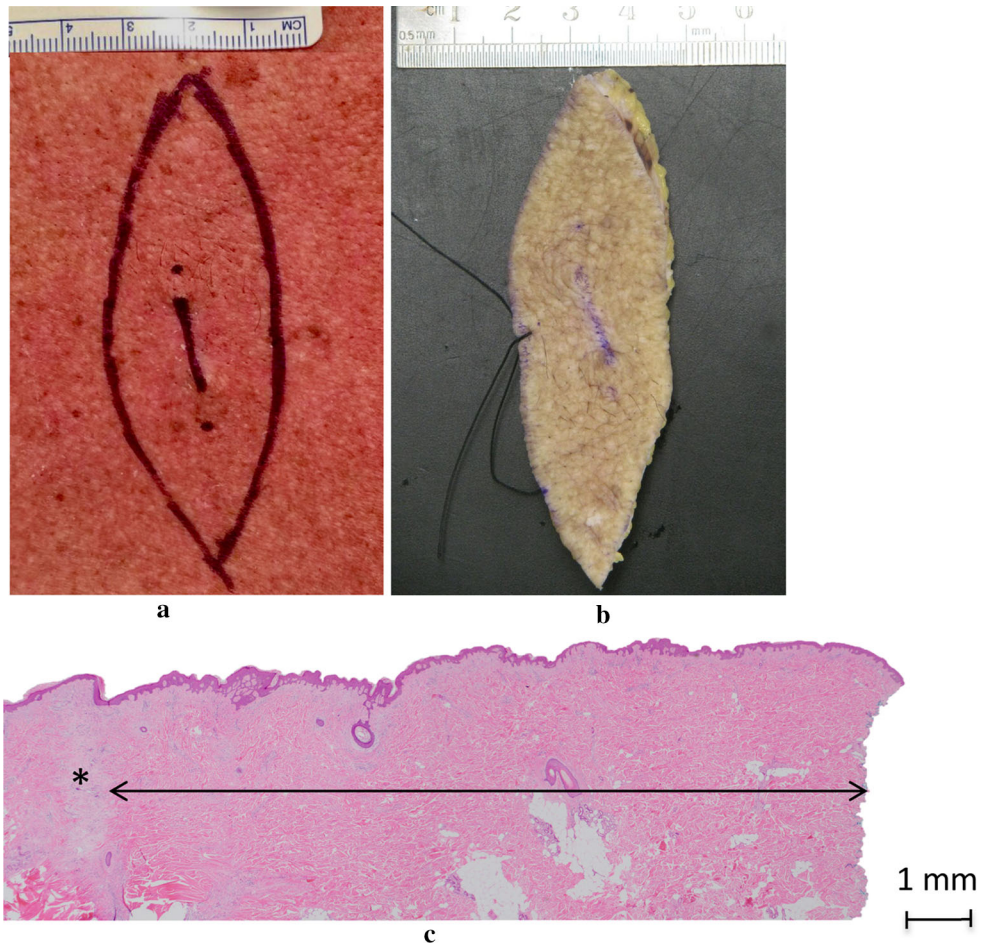

FIG. 1 Wide excision of a melanoma excision biopsy site from the back of a 40-year-old male. The initial diagnostic excision biopsy had indicated a melanoma 1.3 mm in Breslow thickness. **a** A 1.5 cm surgical margin was measured and marked, and the in vivo width was measured. **b** The ex vivo width was measured after formalin fixation. **c** The histologic margin was measured from the edge of the scar to the excision margin of the specimen. * denotes scar

predictable from the in vivo width (Fig. 2). The degree of shrinkage was not significantly affected by patient age, sex, BMI, or anatomic tumor location ($p > 0.05$, Fig. S1).

A measured surgical margin was recorded for 233 specimens; in 87% of specimens, this was greater than the histologic margin. In 6% of specimens, the surgical margin was less than the histologic margin, and, in 7%, the two were equal. The incomplete excision rate was 1.3% ($n = 3$). Histologic margins were, on average, 37% less than the measured surgical margin (95% CI 29–44%). However, there was a significant degree of variability in the rate of shrinkage, and the measured surgical margin was not a strong predictor of histologic margin ($R^2 = 0.55$) (Fig. 3). There was no significant impact on margin discrepancy of clinical or pathological characteristics, including patient age, sex, BMI, anatomic tumor location, tumor subtype, or presence of remaining tumor ($p > 0.05$) (Fig. 4).

DISCUSSION

Wide excision of the primary tumor is the universally acknowledged standard treatment of clinically localized melanoma. However, the definition of 'wide' has changed over the years, based on the results of several prospective clinical trials[2–6]; however, these practice-changing studies have focused on establishing optimal surgical excision margins by correlating the in vivo surgical margin with recurrence and survival, and have not investigated the relationship between surgical margins and the achieved pathologic margins.

In this study, we found that cutaneous tissue specimens have a mean shrinkage rate of approximately 14% for all patients, independent of age, sex, BMI, or anatomic site. It is widely accepted that the elastic and retractile properties of skin result in a degree of shrinkage following excision, but findings from previous studies are lacking in uniformity. A number of studies measured specimen width at

TABLE 1 Clinical and pathologic characteristics of the study cohort

Variable	
Sex (n = 240)	
Female	94 (39.2)
Male	146 (60.8)
Age, years (categorized for analysis; n = 240)	
≤ 55	61 (25.4)
56–67	63 (26.2)
68–75	58 (24.2)
> 75	58 (24.2)
BMI (categorized; n = 240)	
Underweight (< 18.5)	3 (1.2)
Healthy (18.5–24.9)	73 (30.4)
Overweight (25–29.9)	78 (32.5)
Obese (≥ 30)	62 (25.9)
Unknown	24 (10)
BMI categories for analysis (n = 240)	
< 25	76 (31.7)
≥ 25	140 (58.3)
Unknown	24 (10)
Tumor subtype (n = 252)	
Melanoma in situ/APH	74 (29.4)
Invasive melanoma	136 (54)
NMSC	42 (16.6)
Anatomic site (n = 252)	
Torso	90 (35.7)
Extremity	87 (34.5)
Elbow/knee	12 (4.8)
Head/face/scalp	53 (21.0)
Neck	10 (4.0)
Residual tumor (n = 252)	
Yes	94 (37.3)
No	158 (62.7)
Breslow thickness (for invasive melanomas only; n = 136)	
≤ 1	74 (54.4)
1.1–2	40 (29.4)
2.1–4	11 (8.1)
> 4	11 (8.1)
Ulceration (for invasive melanomas only; n = 136)	
Absent	119 (87.5)
Present	16 (11.8)
Unknown	1 (0.7)

Data are expressed as n (%)

BMI body mass index, *NMSC* non-melanoma skin cancer

three timepoints—in vivo, ex vivo immediately after excision, and after formalin fixation. Golomb et al.[13] reported shrinkage of approximately 20% in 199 formalin-fixed cutaneous specimens, and reported that patient age was an independent predictor of shrinkage, with an inverse correlation. Of the total shrinkage, most (92.4%) occurred prior to tissue fixation. In a study of 97 cutaneous specimens, Kerns et al.[15] found a mean shrinkage of length of 20.7% ± 2.15%, but a mean shrinkage of width of 11.8% ± 2.35%, which was slightly less than our finding of 14% shrinkage of specimen width. Kerns et al. also noted that the majority of tissue shrinkage occurred immediately after excision (prior to fixation), and concluded that shrinkage is mainly the result of intrinsic tissue contractility, and not due primarily to formalin fixation. As in the study by Golomb et al., age was reported to be a strong predictor of the proportional amount of shrinkage, with a decrease in the amount of shrinkage of 0.3% per year. Specimens from the trunk displayed 5% more shrinkage than those from the head or neck. Hudson-Peacock et al.[16] examined the relationships between skin excision, wound, and specimen sizes, and reported that formalin-fixed specimens were 31% smaller than the planned excision size, with 22% shrinkage occurring after excision and before fixation. While the degree of shrinkage was significantly higher than in the present study, no effects of sex, site, or age were observed, as we also found.

In our study, we made only two measurements of specimen width—in vivo, prior to surgical excision, and ex vivo, after formalin fixation. While it is interesting that previous studies have explored the relationship between tissue shrinkage, inherent contractility, and formalin fixation, our focus was on the finding with the greatest clinical relevance, i.e. how the specimen evaluated by the pathologist correlated with clinical management (i.e. the surgical specimen). Additionally, we chose to measure only specimen width and not length, as, conventionally, the width of the specimen reflects the minimum planned surgical margin, whereas the planned excision specimen is usually elongated in the long axis to facilitate closure of the surgical wound.

It is of interest that two prior studies[13,15] found an association between age and the degree of shrinkage, with specimens excised from older patients showing less shrinkage. In our relatively large sample size, we did not find a significant impact of age on specimen or margin shrinkage. It is possible that our study, which examined specimens from an Australian population, specifically a population from the state of New South Wales (NSW), may differ from prior studies performed in the United States because of the presence of more severe/extensive baseline solar damage, even among younger individuals. Consistent with this hypothesis, Kerns et al.[15] reported that solar damage leads to less skin contractility, with a greater degree of solar elastosis in specimens that showed decreased contractility. A small study that examined the effect of tretinoin in the treatment of photodamaged skin found a significantly higher degree of solar elastosis in the

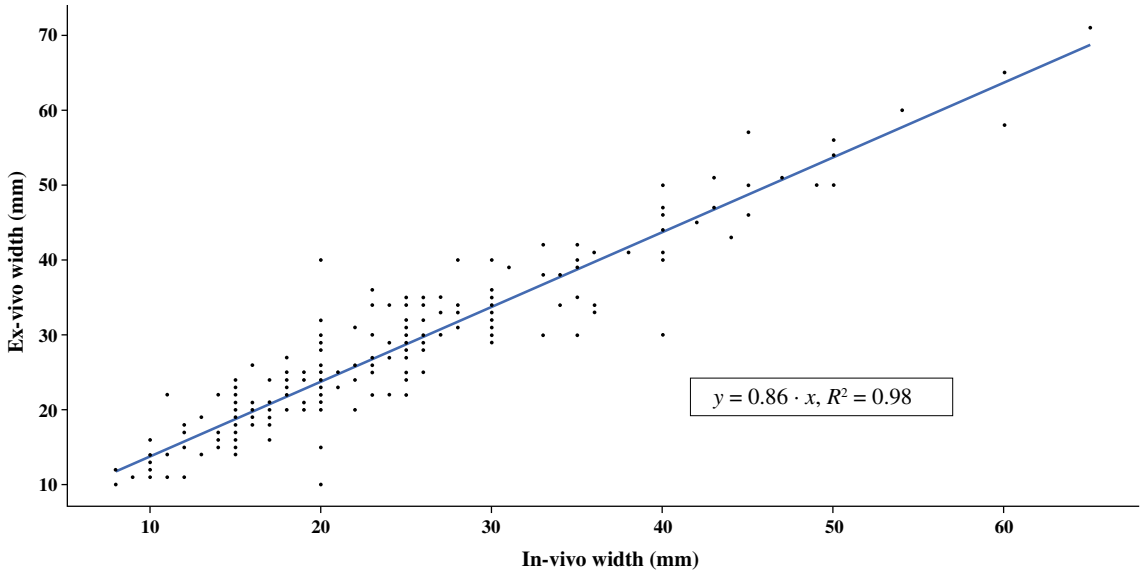

FIG. 2 Correlation between measured in vivo specimen width and formalin-fixed ex vivo specimen width

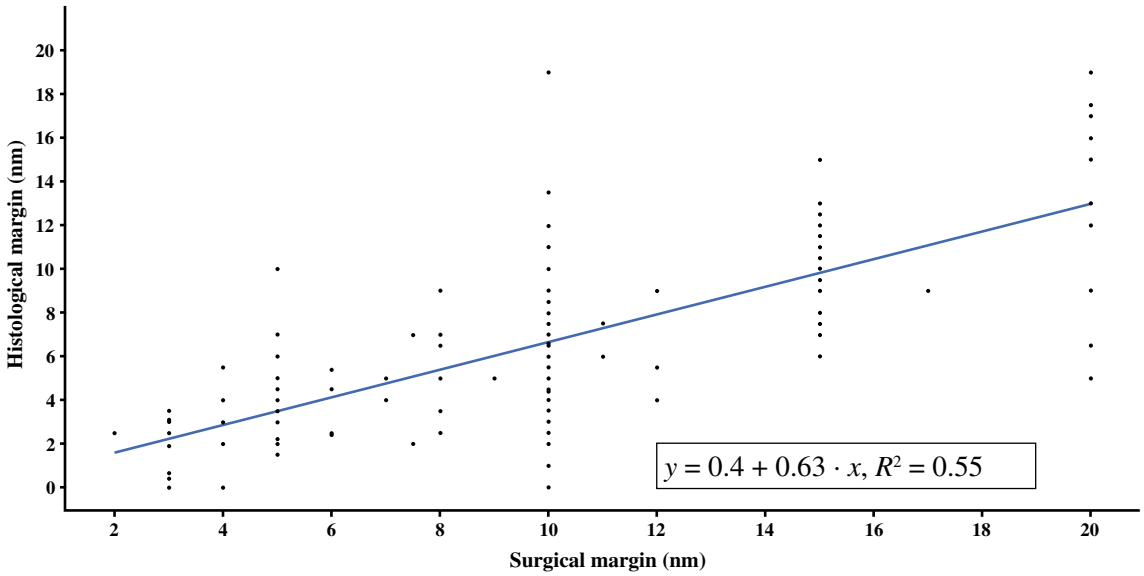

FIG. 3 Correlation between measured surgical margin and histologic margin

skin of patients from Sydney and Newcastle (in NSW), compared with patients from Melbourne, in the more southerly located state of Victoria.[17] The likely etiology, an increase in ultraviolet (UV) radiation in locales closer to the equator, is also the probable explanation for the gradient in the incidence of melanoma and other skin cancers as latitudes approach the equator.[18]

In 233 specimens, we found that the surgical margin measured in vivo was not a good predictor of the histologic margin measured in the formalin-fixed specimen. The high variability in the surgical/histologic margin correlation could not be attributed to any of the recorded clinical or pathologic factors, including age, sex, BMI, anatomic tumor location, tumor subtype, or presence of remaining tumor. This is not in line with the limited existing data. Clausen and Brady[19] reviewed 202 melanoma patients treated at the Memorial Sloan Kettering Cancer Center to determine the discrepancy between intended clinical margin and measured pathologic margin. All patients had prior excision biopsies. They found a median percentage discrepancy between clinical and pathologic margins of 10%, with no significant impact of tumor or patient features. Golomb et al.[13] derived a formula to calculate pre-excision surgical margins from fixed tissue measurements using a correction factor for age. Application of this formula in a larger cohort of 407 specimens led to minor revisions,

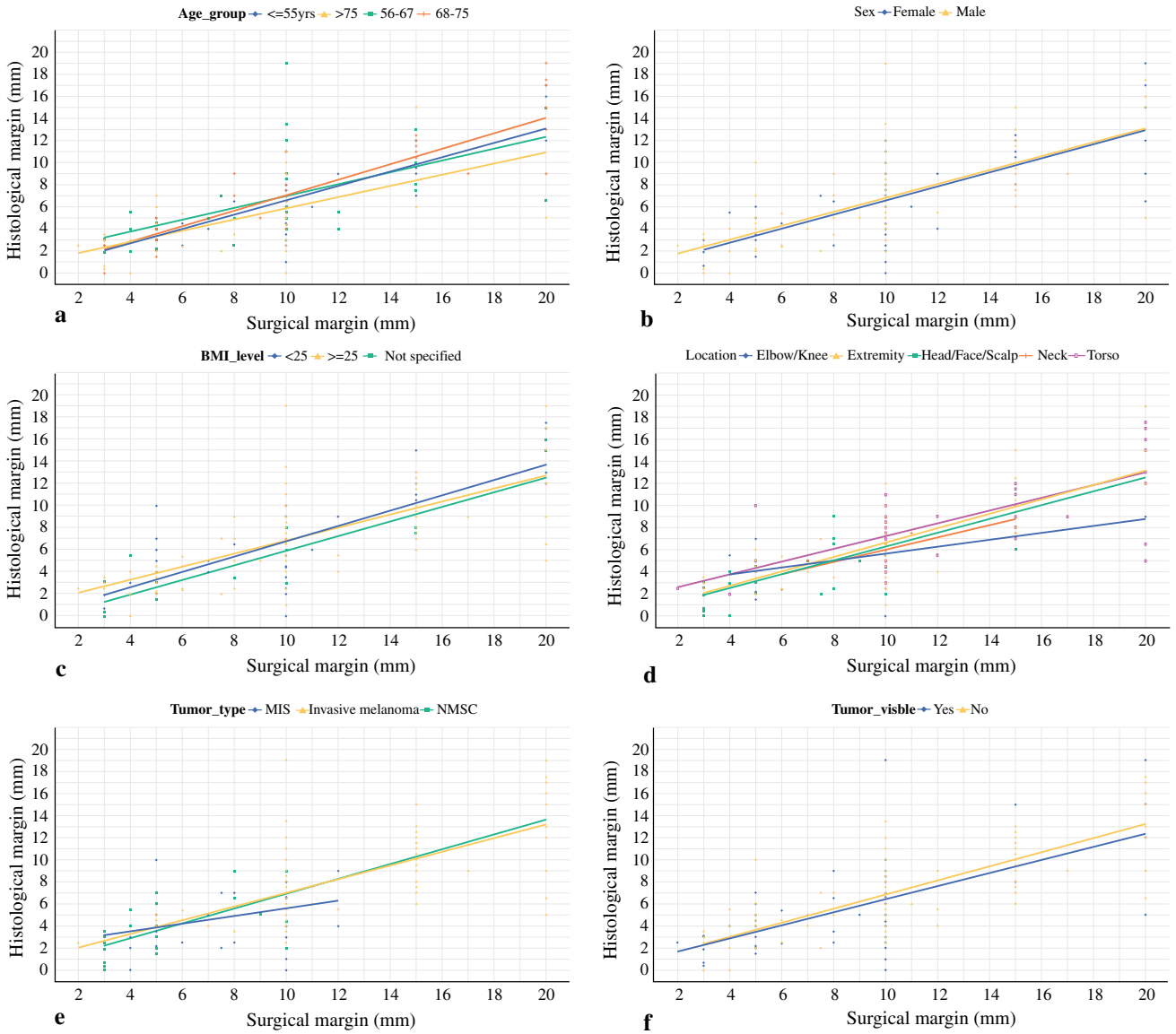

FIG. 4 Correlation between measured surgical margin and histologic margin as a function of **a** patient age; **b** sex; **c** BMI; **d** anatomic site of tumor; **e** tumor subtype; and **f** presence of visible tumor. *BMI* body mass index, *NMSC* non-melanoma skin cancer

which improved accuracy in calculating the pre-excision surgical margins, with 86.5% of cases in the verification group having a calculated pre-excision surgical margin within 3.5 mm of the true measured margin.[14] Both the discovery and validation cohorts included excision specimens containing intact lesions and scars, but with more of the former.

There are many possible reasons for the poor correlation between measured surgical and histologic margins that was found in our study. First, it is common for there to be subclinical tumor extension, especially the in situ component of a melanoma.[20] We hypothesized that this may have been more likely when there was evidence of residual tumor within the excision specimen; however, its presence did not significantly impact the correlation between clinical and histologic margins. Second, there is recognized inter-observer variation between pathologists, particularly when assessing margins for melanoma in situ of lentigo maligna type.[21] Also likely to contribute to the variability in results is inherent imprecision in surgical excision. The intention of all surgeons was to cut precisely on the outside of the marked line (usually approximately 1 mm in width); however, even a slight deviation from the marking, or minor inexactitude of the pre-excision measurement, may result in a substantial difference in the microscopic margin measurement. Additionally, seven surgeons contributed specimens for evaluation, compared with either a single or significantly fewer surgeons in other series.[13,14,19] While

no statistically significant difference was found in margin discrepancy between surgeons in previous studies,[19] it is possible that inter-surgeon variability contributed to the margin discrepancy we found. However, the majority of surgical excisions were performed by a single surgeon (n = 172, 68.3%) and the individual contributions of the other six surgeons were therefore too small to allow a meaningful analysis of inter-surgeon variability to be undertaken. The fourth and likely most significant factor is the irregular and poorly defined microscopic margin of dermal scars (Fig. 1c). Compared with other studies investigating margin discrepancy,[13,14] ours contained a higher proportion of scars compared with specimens with residual tumors; however, while this factor may have impacted the findings of the study, it is of limited clinical importance in specimens where no residual tumor remains.

The importance of achieving adequate pathologic margins to minimize local recurrence has been underscored by several previous studies from MIA. Although local recurrence was an uncommon event, rates were significantly higher if a 'critical threshold' of histologic margin was not met. In patients with primary cutaneous melanomas $\leq$ 1 mm,[9] 1–2 mm,[10] or $\leq$ 2 mm thick,[11] histologic margins $<$ 8 mm resulted in a higher rate of local recurrence. Similarly, in patients with thick melanomas ($>$ 4 mm), histologic margins greater than a 'critical threshold' of 16 mm were associated with better local control.[12] Indeed, in this group of patients, the data suggested a risk reduction in the rate of local recurrence of 9% for each millimeter increase in histologic excision margin. While these data support currently recommended wide excision margins for treating primary cutaneous melanomas, they all assume that the defined 'critical thresholds' of 8 and 16 mm correspond to 1 and 2 cm in vivo surgical margins, respectively, based on an assumed 20% shrinkage rate of formalin-fixed specimens.

However, data from the present study show a consistent shrinkage rate of 14%. Considering any value within the 95% CI range as a plausible shrinkage rate, and considering ease of clinical applicability, it would be reasonable for a clinician to anticipate specimen shrinkage of approximately 15%. Perhaps more relevant to the surgeon is our finding that the correlation between a measured surgical margin and the histologic margin is highly variable. This suggests that it is difficult to predict a histologic clearance margin from the planned surgical excision, and raises the important question of whether clinical practice guidelines should be revised to require satisfactory microscopic clearance margins, rather than simply specifying surgical margins measured in vivo.

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