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Shrinkage of skin excision specimens: formalin fixation is not the culprit

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Summary

Background Discrepancies between cutaneous specimen sizes reported by the dermatosurgeon and the pathologist are important to evaluate because of their legal implications for malignant tumours and the downcoding of surgical acts.

Objectives The objective of this study was to determine the magnitude of changes in size and the factors influencing the retraction of routine skin excision specimens.

Methods Three measurements of 82 skin excision specimens—consisting of length and width of the planned surgical excision (*in vivo*), length, width and depth of the specimens following excision (*ex vivo*) and of the specimens after formalin fixation (*in vitro*)—were performed and compared using a nonparametric paired test. Factors (age, sex, type and location of the lesions and initial measures) that could influence the amount of shrinkage were analysed using multiple linear regression models.

Results The mean *in vivo* to *in vitro* shrinkage was 16% for length and 18% for width ($P < 0{\cdot}001$). The shrinkage was significant between *in vivo* and *ex vivo* measures ($P < 0{\cdot}001$), while no difference was observed between *ex vivo* to *in vitro* measures. In multivariate analysis, length shrinkage increased significantly with initial length (regression coefficient of 0·24, $P = 0{\cdot}001$) and limb location (1·25, $P = 0{\cdot}048$), and decreased significantly with initial width (−0·19, $P = 0{\cdot}016$). After adjusting for initial width, width shrinkage was neither significantly associated with type of lesion (malignant or not, $P = 0{\cdot}20$), nor with location ($P = 0{\cdot}35$).

Conclusions Shrinkage of skin excision specimens occurred immediately after surgical excision and prior to formalin fixation. Patients' age, sex and type of skin lesion did not influence the amount of shrinkage. Length shrinkage was more important for specimens excised from the extremities and increased with initial length and smaller width.

Excised skin is known to shrink when removed, in relation with its retractile properties, and it is commonly accepted that formalin fixation itself induces retraction of the samples.[1] Discrepancies between surgical and pathological reported specimen sizes are important to evaluate because of their legal implications, in term of respect of surgical margins when excising malignant tumours and downcoding of surgical acts. Indeed, if the *in vivo* and excised fixed tissue dimensions are significantly different, assessment of tumour margin clearance from fixed skin specimens would be misleading. The shrinkage phenomenon is widely recognized and has already been studied.[2–4] However, results of these previous studies are inconclusive on the amount of shrinkage on the one hand and on the potential role of formalin fixation on the other. Moreover, several factors such as sex, age and location of the lesion have been reported to influence the extent of shrinkage, but there again results were controversial. Therefore, the objectives of this prospective study were (i) to evaluate the degree of shrinkage occurring in cutaneous specimens from the time prior to surgical excision to the time after specimen fixation; (ii) to determine when (before and/or after formalin fixation) the shrinkage occurs; and (iii) to assess the role of factors suspected to be associated with shrinkage of skin excision specimens.

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Patients and methods

The study was conducted prospectively between 3 January and 3 April 2007 in one unit of plastic surgery with four plastic surgeons. Patients planned to have a surgical excision for a suspected malignant skin tumour (melanocytic tumour, basal or squamous cell carcinoma) or a skin lesion requiring a full-thickness skin excision down to fat or deep fascia were eligible. All eligible consecutive patients having surgical excision on a Tuesday or Thursday during this 3-month period were included. All skin excision specimens were sent to the same department of pathology and analysed by two of us (S.F. and J.N.D.).

Skin measurements and data collection

All skin measurements were performed by one of us (J.N.D.). The planned excision was outlined with a pen. The length and width of the planned excision were measured—before local anaesthetic (2% lidocaine with 1 : 80 000 epinephrine) injection—using a millimetre ruler. These measurements determined immediately prior to excision were defined as the *in vivo* data. Length, width and thickness of each specimen were also measured immediately after excision, along the same axes, defining the *ex vivo* data; and then, after 4% formalin fixation for 24–48 h, determining the *in vitro* data.

Skin measurements, and also patients' characteristics and clinical factors that may influence skin plasticity and skin shrinkage, were recorded prospectively with a standardized form and are listed in Table 1.

Statistical analyses

Eighty-two patients having 97 skin lesions were included in the study. When several lesions were excised for one patient, only one lesion was randomly selected and analysed.

Table 1 Characteristics of the 82 patients

	n	%
Female sex	53	64·6
Age, years	37·9 (± 22·6)	
Skin location		
Head and neck	34	41·5
Trunk	27	32·9
Limbs	21	25·6
Malignant lesion	14	17·1
Initial measurements, mm		
In vivo length	18·8	± 13·4
In vivo width	12·1	± 7·6
Ex vivo depth	5·9	± 2·8
Delay from fixation to *in vitro* measurements, h		
≤ 24	53	64·6
24–28	19	23·2
> 48	10	12·2

Assessment of shrinkage

The *in vivo* length and width measurements of the skin specimens were compared with the *in vitro* measures by using the paired nonparametric Wilcoxon-signed rank test; in case of significant differences, two by two analyses (i.e. *in vivo* vs. *ex vivo* and *ex vivo* vs. *in vitro*) were performed in order to detect when exactly the shrinkage occurred. The *ex vivo* skin specimens' depth was compared with the *in vitro* measurement.

Factors potentially associated with shrinkage

When significant differences were evidenced between successive measurements, the extent of shrinkage was estimated by the differences observed between values, e.g. length *ex vivo* shrinkage = (*in vivo* length − *ex vivo* length) and length *in vitro* shrinkage = (*ex vivo* length − *in vitro* length). These differences were compared between sexes, age classes, types of skin lesion (benign or malignant) and skin location of the initial lesion by using the nonparametric Kruskal–Wallis test. For that purpose, age was categorized by quartiles; cut-off points of 50 and 60 years, which were previously found to be associated with shrinkage, were also analysed.[2,3] The correlation between age and shrinkage was also assessed by the Spearman's rank correlation. Potential associations between the extent of the shrinkage and initial measurements were assessed by using the Spearman's rank correlation.

Then, multiple linear regression models were used to take into account simultaneously all factors associated with shrinkage in univariate analysis ($P < 0{\cdot}10$).

Quantitative variables are reported as mean (SD) or median (interquartile range) as appropriate. Categorical variables are reported as number (%).

All tests were two-tailed, a P-value of $\leq 0{\cdot}05$ was considered to indicate statistical significance. Data were analysed using the Stata Statistical software (release 8.0, 2003; StataCorp, College Station, TX, U.S.A.).

Results

Characteristics of the 82 patients are described Table 1. Of these 82 patients, 29 were male and 53 were female. Their mean age was 37·9 years (± 22·6), ranging from 1 to 89 years. The 82 skin lesions analysed were as follows: 63 melanocytic tumours, 10 basal cell carcinomas, one squamous cell carcinoma and eight other skin lesions.

Comparisons of the repeated measurements

Length and width of skin specimens significantly decreased between planned excision (*in vivo*) and measurements after formalin fixation (*in vitro*) (Table 2). The mean relative shrinkage was 16·0% for length and 18·2% for width. The shrinkage between *in vivo* and *ex vivo* measurements was significant for both length and width ($P < 0{\cdot}0001$). On the contrary, there was no significant difference between *ex vivo* and *in vitro*

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Table 2 Comparisons of the 82 skin specimens dimensions, before (*in vivo*), immediately after excision (*ex vivo*) and after formalin fixation (*in vitro*)

Skin specimen dimensions[a]	Skin measurements			Total shrinkage		Differences between measurements			
				In vivo to *in vitro*		*In vivo* to *ex vivo*		*Ex vivo* vs. *in vitro*	
	In vivo	*Ex vivo*	*In vitro*	Differences	P-value[b]	Differences	P-value[b]	Differences	P-value[b]
Length	18·8 (13·4)	15·6 (11·7)	15·8 (11·7)	4·1 (3·4)	**< 0·001**	4·2 (3·2)	**< 0·001**	−0·2 (1·4)	0·54
Among 14 malignant lesions	31·6 (16·8)	27·6 (15·6)	26·9 (15·6)	4·7 (3·3)	**0·001**	4·0 (2·5)	**0·001**	−0·7 (1·9)	0·19
Width	12·1 (7·6)	9·9 (6·6)	9·9 (6·4)	2·2 (2·4)	**< 0·001**	2·2 (1·9)	**< 0·001**	−0·04 (1·4)	0·81
Among 14 malignant lesions	20·6 (10·3)	17·7 (9·6)	17·3 (9·3)	3·4 (1·7)	0·001	−2·9 (1·2)	0·001	0·04 (1·1)	**0·10**
Depth (n = 42)	–	5·9 (2·8)	5·1 (2·4)	–		–		0·8 (1·3)	**0·0006**

[a]Data are expressed as mean (SD) (mm). [b]P-value of the paired Wilcoxon-signed rank test. *Italics*, data relating to malignant lesions; **bold**, P-value is significant.

dimensions, both for length (P = 0·54) and width (P = 0·81) (Table 2) even when accounting for formalin fixation duration (≤ 24 h vs. > 24 h) (data not shown). Similar results were observed when we considered only the 14 malignant lesions. Interestingly, a significant difference was assessed between the *ex vivo* and *in vitro* specimen depth (P = 0·0006).

Factors potentially associated with shrinkage

In univariate analysis, length shrinkage was not significantly associated with sex, age of patient or type of skin lesion (Table 3). No association with age was observed when considering cut-off points of 50 and 60 years (data not shown) or when using correlation analysis. The extent of *in vivo* to *ex vivo* length shrinkage was significantly associated with location, shrinkage being important for trunk and limb lesions. The extent of *in vivo* to *ex vivo* width shrinkage was significantly more important for malignant lesions (P = 0·02); a trend for an association was observed for limb location (P = 0·056).

Strong positive correlations were observed between initial measurements (*in vivo* length, width and *ex vivo* depth) and the extent of length and width shrinkage (Table 3).

Depth shrinkage was significantly associated only with *ex vivo* depth (rho = 0·51, P = 0·001).

In multivariate analysis, length shrinkage significantly increased with initial length (regression coefficient of 0·24, P < 0·001) and limb location (1·25, P = 0·048), and significantly decreased with initial width (−0·19, P = 0·016)

Table 3 Relations between length (and width) shrinkage and characteristics of patients, location of skin lesion and initial measurements

	Shrinkage: *in vivo* to *ex vivo* difference			
	Length	P-value[a]	Width	P-value[a]
Sex				
Male	4 (2–7)	0·38	3 (1–4)	0·18
Female	3 (2–5)		2 (1–3)	
Age, years[b]	rho = −0·11	0·33	rho = 0·03	0·79
≤ 25th (22 years) (n = 21)	3 (2–7)	0·50	1 (1–4)	0·25
25th to 50th (22–37 years) (n = 21)	4 (3–6)		3 (1·5–3)	
50th to 75th (37–56 years) (n = 20)	3 (2–4·5)		1 (0·5–2)	
> 75th (> 56 years) (n = 19)	3 (2–5)		2 (1–4)	
Type of skin lesion				
Benign skin lesions (n = 68)	3 (2–5)	0·88	1·5 (1–3)	**0·02**
Malignant skin tumour (n = 14)	3 (2–6)		3·5 (2–4)	
Skin location				
Head and neck (n = 34)	3 (2–4)	**0·007**	1 (1–2)	**0·056**
Trunk (n = 27)	5 (3–8)		3 (1–4)	
Limbs (n = 21)	4 (2–5)		2 (0–3)	
Initial measurements in mm[b]				
In vivo length	rho = 0·48	**<0·001**	rho = 0·51	**<0·001**
In vivo width	rho = 0·33	**0·02**	rho = 0·68	**<0·001**
Ex vivo depth	rho = 0·39	**0·001**	rho = 0·35	**0·004**

Continuous data are reported as median (interquartile range) except when otherwise indicated. [a]P-value of the Kruskall–Wallis test except when otherwise indicated. [b]Spearman's rho coefficient, P-value of the Spearman's rank correlation. **Bold**, P-value is significant.

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Table 4 Multivariate analysis of factors associated with length *in vivo* to *ex vivo* shrinkage (multiple linear regression analysis)

	In vivo to *ex vivo* shrinkage; median value (IQR)	Regression coefficient; 95% CI	P-value[a]
Skin location			
Head and neck	−3 (−2 to −4)	Ref category	
Trunk	−5 (−3 to −8)	1·04 (−0·26 to 2·35)	0·12
Limbs	−4 (−2 to −5)	1·25 (0·1–2·57)	**0·048**
Initial length			
≤ 25th (12)	−2·3 (−2 to −3)	0·24 (0·15–0·33)	**< 0·001**
25th to 75th (12–23)	−3 (−2 to −5)		
> 75th (23)	−7 (−3 to −9)		
Initial width			
≤ 25th (7)	−1 (0 to −1)	−0·19 (−0·35 to −0·034)	**0·016**
25th to 75th (7–14)	−2 (−1 to −3)		
> 75th (14)	−4 (−3 to −4)		

IQR, interquartile range; CI, confidence intervals. [a]P-value of multiple linear regression analysis, for this analysis continuous variables were not categorized. **Bold**, P-value is significant.

(Table 4). After adjusting for initial width, width shrinkage was not significantly associated with type of lesion (malignant or not, $P = 0·20$) or with location ($P = 0·35$).

Discussion

Our study showed that skin specimens predictably shrink after excision and formalin fixation by approximately 16% in length and 18·2% in width. We demonstrated that formalin fixation was not the culprit, as the shrinkage was significant between *in vivo* and *ex vivo* measures ($P < 0·001$), while no difference was observed between *ex vivo* to *in vitro* measures. The amount of shrinkage was not influenced by the patient's sex or age. The initial size of the planned excision (*in vivo* length and width) and limb location were the only factors associated with length shrinkage in multivariate analysis. Width shrinkage was associated only with initial width.

The number of skin specimens prospectively analysed in our study ($n = 82$) is higher than in previously published works (i.e. 54 for Gregory *et al.*).[2] However, of the 82 skin lesions we analysed, only 14 (17%) were malignant lesions. Our study differs from previous reports, which displayed 46–100% malignant lesions.[2–4] Our small number of malignant skin lesions is a limit, which may partly explain the lack of association between shrinkage and type of lesions. Although we tested several factors that could potentially affect the amount of shrinkage in multivariate analysis, we cannot exclude that other unknown confounders may have affected our results. However, only one of the three series previously published analysed potential confounders by using multivariate models.[3]

Our results, indicating a shrinkage of 16% in length and 18% in width, are consistent with previously published results as a shrinkage of 22% was reported by Gregory *et al.* and 20·7%, by Golomb *et al.*[2,3] On the contrary, a more important shrinkage, namely 31%, has been noticed by Hudson-Peacock *et al.*[4] We demonstrated that shrinkage of skin excision specimens occurs immediately after surgical excision and prior to formalin fixation, so that formalin fixation does not contribute at all to specimen contraction. This is consistent with some but not all previously published results. Indeed, according to Golomb *et al.*, of the total amount of shrinkage that occurred in excised skin specimens, 94·2% occurred immediately after surgical excision and prior to tissue fixation.[3] For Hudson-Peacock *et al.* also, most (22%) of the 31% shrinkage occurred after excision and before fixation.[4] On the other hand, for Gregory *et al.*, the total amount of shrinkage resulted from both post-excisional shrinkage and formalin fixation, the two of them being equally implicated.[2]

Our results indicate that the location of the lesion on the limbs is associated with a more significant length shrinkage, in comparison with the location on the head and neck region, whereas sex and age of patients do not influence shrinkage. The association between trunk location and the extent of length shrinkage we observed in univariate analysis disappeared after adjustment for initial size. Influence of location, sex and age is variably assessed in the literature. Indeed, Hudson-Peacock *et al.* did not observe any sex, age or site effects on the amount of shrinkage.[4] According to Gregory *et al.*, the degree of skin shrinkage was related to age and location as significantly less skin shrinkage occurred in individuals older than 60 years and on the head and neck.[2] He attributed this phenomenon to a loss of tensile strength with age and increased photodamage. Nevertheless, only univariate statistical analysis was performed; initial size was not taken into account. Finally, for Golomb *et al.*, the only factors that proved to contribute significantly to shrinkage were the patient's age (with a greater degree of shrinkage under 50 years) and the diameter of the *in vivo* specimen, using multiple regression analysis.[3] Other factors such as sex and anatomical location were not shown to influence the degree of shrinkage.

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This study shows that significant differences exist between planned excisions and fixed specimen sizes, with a 16% shrinkage in length and an 18% shrinkage in width. The amount of shrinkage is variably influenced by location of the lesion, namely enhanced for lesions excised from the limbs but not influenced by patient's age or sex. This shrinkage is responsible for the underestimation of the true specimen size and of the surgical margins when measurements are realized *in vitro*, macroscopically and/or histologically on fixed specimens. To our knowledge, this is the first study that reveals a more important shrinkage for skin specimens excised from the limbs, independently of initial size. Besides, it emphasizes that the shrinkage of skin specimens occurs immediately after surgical excision and prior to formalin fixation, possibly in relation to skin elasticity and therefore that the fixative is not the culprit.

It is very important to quantitatively evaluate the discrepancies between surgical and pathological specimen sizes. Indeed, on the one hand, they could lead to claims related to downcoding of surgical acts, underpayment and loss of revenue for surgical dermatologists and plastic surgeons. More importantly, it is of concern that the assessment of tumour margin clearance from a fixed specimen is misleading because the *in vivo* and excised fixed tissue dimensions differ significantly.

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