Melanocytes/Melanoma Associations of Statins and Diabetes with Diagnosis of Ulcerated Cutaneous Melanoma

Associations of statins and diabetes with diagnosis of ulcerated cutaneous melanoma

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Abbreviations: AIC- Akaike information criterion, AUC ROC- area under the Receiver

Operating Characteristic curve, BMI- body mass index, CI- confidence interval , NSAIDS-

non-steroidal anti-inflammatory drugs, OR- odds ratio, SAS- Statistical Analysis System

ABSTRACT

Ulcerated primary melanomas are associated with an inflammatory tumor micro-environment. We hypothesised that systemic pro-inflammatory states and anti-inflammatory medications are also associated with a diagnosis of ulcerated melanoma. In a cross-sectional study of 787 patients with newly-diagnosed clinical stage IB or II melanoma, we estimated odds ratios (ORs) for the association of pro-inflammatory factors (high body mass index (BMI), diabetes, cardiovascular disease, hypertension and smoking) or use of anti-inflammatory medications (statins, aspirin, corticosteroids and non-steroidal anti-inflammatory drugs), with ulcerated primary melanoma using regression models and subgroup analyses to control for melanoma thickness and mitotic rate. Based on information from 194 patients with ulcerated and 593 patients with non-ulcerated primary melanomas, regular statin users had lower likelihood of a diagnosis of ulcerated primary melanoma (OR 0.67, 95% CI 0.45-0.99) and this association remained after adjusting for age, sex, thickness and mitosis. When analysis was limited to melanomas that were ≤2mm thick and had ≤2 mitoses/mm^2 (40 ulcerated; 289 without ulceration), patients with diabetes had significantly raised odds of diagnosis of ulcerated melanoma (OR 2.90, 95% CI 1.07-7.90), adjusted for age, sex, BMI and statin use. These findings support our hypotheses that statin use is inversely associated, and diabetes is positively associated, with ulcerated melanoma.

INTRODUCTION

Ulceration of a primary cutaneous melanoma signifies a poorer prognosis than a non-ulcerated melanoma of the same thickness and is integral to clinical staging of the disease (Balch et al., 2009). The histopathologic features of tumor thickness, ulceration and mitotic activity are considered the hallmarks of rapidly growing melanomas (Balch et al., 2009, Thompson et al., 2011), hence the presence of ulceration likely reflects a highly proliferative phenotype. Not all thick or highly mitotic lesions are ulcerated however (Balch et al., 2009), and this suggests that processes other than cell proliferation may influence the development of ulceration (Balch et al., 2009, Gogas et al., 2009, Spatz et al., 2010, Thompson et al., 2011).

Neither a patient's age or sex, nor the melanoma's anatomic location or level of pigmentation appear to be associated with ulceration (Balch et al., 1980, Newton-Bishop et al., 2014). However, evidence suggests inflammation in the tumor microenvironment is linked to ulceration (Jewell et al., 2015, Storr et al., 2012, Winnepenninckx et al., 2006) as shown by the greater numbers of macrophages, increased microvessel density and higher expression of genes encoding inflammatory and wound-healing factors in ulcerated lesions (Jewell et al., 2015, Storr et al., 2012, Winnepenninckx et al., 2006). Although targeted immune activation is used therapeutically in melanoma to induce long-term survival in metastatic disease, there is overwhelming evidence that 'smouldering' inflammation in the tumor microenvironment suppresses antitumor activity and aids angiogenesis, cancer cell proliferation, survival and metastasis (Mantovani et al., 2008, Melnikova and Bar-Eli, 2009). 'Smouldering' inflammation has been described as chronic, often subclinical, inflammation involving macrophages, granulocytes and chemical mediators and causing inhibition of T-cell-dependent antitumor activities (Balkwill et al., 2005). Thus while it is clear that a component of unfavourable tumor inflammation is intrinsically driven by oncogenic changes,

it remains largely unknown if factors associated with systemic pro-inflammatory states, may also enhance tumor inflammation and ulceration.

Systemic factors known to cause pro-inflammatory states include obesity, diabetes, hypertension, cardiovascular disease and smoking (van Kruijsdijk et al., 2009). The chronic inflammatory activation observed in obese people is thought to contribute to their higher risk of developing other types of cancer including breast, endometrial, colorectal or liver cancer (Mazzarella, 2015, Sutherland et al., 2004) as well as chronic diseases such as hypertension, diabetes and cardiovascular disease (2010, Herder et al., 2016, Wang et al., 2013, Wang et al., 2016). A prevalent inflammatory state is also observed in smokers as evidenced by quantifiable increases in circulating inflammatory markers (Bakhru and Erlinger, 2005) implicated in the pathogenesis of cardiovascular disease.

Medications with anti-inflammatory properties, such as statins (Ferri and Corsini, 2014), nonsteroidal anti-inflammatory drugs (NSAIDs) (Green, 2001) and corticosteroids (Recio and Fuentes, 2012) are commonly used to reduce the burden of systemic inflammation in patients with imminent or established cardiovascular disease or other chronic conditions and their anti-inflammatory properties may also be relevant to the pathogenesis of inflamed and ulcerated melanomas.

The only published study investigating the relationship between ulceration and systemic inflammatory factors (Newton-Bishop et al., 2014) reported that ulceration was associated with diabetes, smoking, lower vitamin D levels and higher BMI at diagnosis. Although this was a cohort study, all clinical stages including very late stage melanomas were included, so that the observed associations with ulceration could not be distinguished from associations with late diagnosis. ==Thus new evidence is required to clarify if systemic pro-inflammatory factors and medications with anti-inflammatory properties are indeed==

associated with promotion or inhibition of melanoma-genesis respectively and the presence or not of ulceration. If potentially modifiable factors were shown to be associated with melanoma ulceration this would assist in targeted prevention and enable clinicians to advise patients in an informed manner. We therefore explored whether the systemic inflammation linked to smoking, obesity, hypertension, diabetes and cardiovascular disease or alternatively, if the anti-inflammatory properties of medications such as statins, nonsteroidal anti-inflammatory drugs and corticosteroids, are associated with the diagnosis of an ulcerated rather than a non-ulcerated primary melanoma independent of other associated histopathological features of melanoma.

RESULTS

Patient characteristics

Of the 1,254 invited patients, 825 (66%) consented to take part. A further 38 patients were found to be ineligible after consent, leaving 787 study participants (mean age at diagnosis, 62 years; 57% male). Of the 787, 194 (25%) melanomas were reported as showing ulceration on histopathological examination. Participants with ulcerated melanoma were older (56% >65 years), more likely to be male, more likely to have outdoor occupations, and they had thicker (>2mm) rather than thinner (≤2mm) melanomas and higher numbers of mitoses (>6/mm^2) than those with non-ulcerated primary melanomas (all p<0.05) (Table 1).

Pro- and anti-inflammatory factors and ulceration

After adjustment for age and sex, regular statin users were significantly less likely to be diagnosed with an ulcerated melanoma compared to nonusers (OR 0.67, 95% CI 0.45-0.99) and this inverse association remained strong and significant after further adjustment for thickness and mitosis (OR 0.64, 95% CI 0.42-0.98) (Table 2). Similarly, regular aspirin, NSAIDs and corticosteroid use all tended towards inverse associations with ulceration (Table

2). Patients using at least one of either statins, aspirin or NSAIDs regularly, were less likely to be diagnosed with an ulcerated melanoma when adjusted for age, sex, thickness and mitotic rate (OR 0.68, 95% CI 0.46-1.00) (Table 2). Pro-inflammatory factors (obesity, smoking, hypertension, diabetes and cardiovascular disease) were not associated with ulceration in this analysis.

We used three model selection techniques to determine which patient features, histopathological and/or inflammatory factors were associated with melanoma ulceration. These model selection approaches were considered due do their diverse criteria and their different abilities to produce a set of efficient, parsimonious statistical predictors. Using an exhaustive model selection approach, thickness, mitosis, regression, aspirin use and weekend sun exposure best predicted ulceration status (best AIC: 665). This model produced an area under the ROC curve of 0.74 (95% CI 0.70-0.79), indicating good discriminative ability. When utilising a lasso model selection technique, age, sex, thickness, mitotic rate, statin use and BMI were selected as best fit, with an AIC value of 604 and AUC ROC curve=0.75 (95 % CI 0.70-0.79). The same parameters were obtained using a stepwise regression approach, with the addition of corticosteroid use AUC ROC curve = 0.74 (95 % CI 0.70-0.79). (Table S1).

Subgroup analysis

Amongst the subgroup of melanomas with relatively low proliferation (≤2mm thick and ≤2/mm^2 mitotic rate), 40 (12%) were ulcerated and 298 not ulcerated. After adjustment for age and sex, diabetes increased the odds of ulcerated melanoma (OR 2.89, 95% CI 1.26-6.60) and this association remained when adjusted further for BMI and statin use (OR 2.90, 95% CI 1.07-7.90) (Table 3). Overweight or obesity, hypertension and heart disease were positively and use of statins, aspirin or NSAIDs were inversely associated with ulceration, but none significantly (Table 3). The magnitude of the association between regular statin use

and ulcerated melanoma in the subgroup analysis (Table 3) was very similar to that obtained in the overall analysis, although again it was not significant.

DISCUSSION

There is clear evidence that the tumor microenvironments of most melanomas are inflamed, but ulcerated melanomas are associated with especially high levels of tumor inflammation (Jewell et al., 2015, Melnikova and Bar-Eli, 2009, Winnepenninckx et al., 2006) with high density of macrophages, microvessels (Jewell et al., 2015, Storr et al., 2012) and enhanced expression of the genes encoding inflammatory and wound-healing factors (Jewell et al., 2015, Winnepenninckx et al., 2006). In this cross-sectional study of localised primary melanomas at high risk of recurrence, we explored whether factors that are believed to modify the level of systemic inflammation, may also be associated with melanoma ulceration status. Our primary finding suggests that statin users have a substantially decreased likelihood of being diagnosed with an ulcerated melanoma compared with non-users after adjusting for age, sex, thickness and mitotic rate. Other medications with anti-inflammatory properties (aspirin, NSAIDs and corticosteroids) also demonstrated strong inverse relationships with ulcerated melanomas.

As other characteristics of fast-growing tumors, namely increased thickness and mitotic rate, are highly associated with tumor ulceration, we removed their influence in a subgroup analysis of patients who had melanomas without these features. Within this subgroup, diabetics were more than twice as likely to be diagnosed with ulcerated than non-ulcerated melanoma. While we did not observe any other significant association with ulceration in this smaller subgroup, it is notable that, free of confounding by thickness >2mm and mitotic rate $>2/mm^2$, positive and inverse associations were seen as hypothesised with all

pro-inflammatory and anti-inflammatory factors respectively that could be assessed, and the strong inverse association between ulcerated melanoma and statins remained, albeit no longer significant in the smaller sample.

While statins are traditionally used as cholesterol-lowering medications, they are becoming increasingly recognised for their anti-cancer effects, regulating tumor proliferation, apoptosis, angiogenesis and metastasis that occur via inhibition of metabolic products of the 3-hydroxy-3-methylglutaryl coenzyme A reductase reaction, including the inhibition of Rho GTPases and Rho dependant signalling pathways (Hindler et al., 2006, Sarrabayrouse et al., 2017). Early epidemiological studies suggested statins may prevent melanoma development (Downs et al., 1998), yet more recent studies do not support this (Bonovas et al., 2010, Curiel-Lewandrowski et al., 2011, Jagtap et al., 2012). There is however growing evidence that statins may reduce melanoma mortality, particularly in males (Livingstone et al., 2014). In our cohort, statin use decreased the likelihood of being diagnosed with an ulcerated versus non-ulcerated melanoma. It is possible that statins modify inflammatory mechanisms involved in tumor ulceration, and that this reduction may, at least in part, account for the reduced melanoma progression and metastasis observed in regular statin users (Dulak and Józkowicz, 2005).

We observed that the other medications with anti-inflammatory properties that we assessed were also negatively associated with ulcerated melanomas when adjusted for thickness and mitotic rate though non-significantly. We therefore suggest that the chemoprotective effects of NSAIDs, aspirin and corticosteroids on tumor ulceration and inflammation also warrant further exploration in larger prospective studies. In addition, the dosage and duration of medication use are thought to be important determinants of potential chemoprotective effects and should be considered in prospective studies. (Cuzick et al., 2009, Rothwell et al., 2010).

In the subgroup of melanomas with relatively low proliferation, diabetic patients were more commonly diagnosed with an ulcerated versus non-ulcerated melanoma and this association remained after adjusting for potential confounding factors. We deduce that we did not observe this association in the overall analysis due to residual confounding by thickness or mitotic rate. This positive association is consistent with results from a recent cohort study also showing a positive association between diabetes and ulceration in univariate analysis (Newton-Bishop et al., 2014). Diabetes is a known pro-inflammatory state, as evidenced by low-grade elevation of circulating markers such as C- reactive protein, sialic acid and pro-inflammatory cytokines. Diabetics have higher incidence rates of primary cancer (liver, pancreas, endometrium, colon, breast and bladder cancer) and micro- and macro-vascular pathology, and there is general consensus that the pathogenesis of these diseases in diabetics is linked to activation of inflammatory pathways (Giovannucci et al., 2010). It is also possible that the higher likelihood of melanoma ulceration in diabetics, as observed in the subgroup analysis, may be mediated by diabetes-induced inflammation. Regular surveillance of diabetic patients at high-risk of melanoma would be clinically indicated, if diabetes were confirmed to increase risk of melanoma ulceration.

Strengths of this study include the epidemiological evidence regarding a possible association between ulcerated melanomas and systemic inflammatory factors that accounts for the potential confounding by melanoma thickness and mitotic rate. Our results are based on a large cohort of localised primary melanomas of >1mm thickness, with detailed clinical and histological data obtained from pathological reports. The major limitation of our study is its cross-sectional design. Also our self-reported medication measures may have been subject to recall error, and missing data decreased the statistical power of some analyses. We did not perform histological evaluation of cases to confirm ulceration status, however inter-observer agreement in reporting melanoma features is high in Australia (Murali et al., 2009).

In summary, we have demonstrated that regular statin users have reduced likelihood of a diagnosis of ulcerated melanoma. Amongst patients with melanomas of relatively low proliferation, it was seen that diabetics also have a higher probability of an ulcerated versus non-ulcerated melanoma. Evidence from larger prospective studies is required to confirm whether these associations between ulcerated lesions and statins and diabetes are causal relationships. Further investigation into the chemoprotective role of other specific anti-inflammatory medications is also warranted.

MATERIALS AND METHODS

Study population

Participants were recruited prospectively between October 2010 and October 2014, from various specialist public Queensland hospital clinics, and private practices of collaborating surgeons. Patients were invited to participate by their treating doctor (or by study personnel with doctors' permission) if they met the following inclusion criteria: histologically-confirmed new diagnosis of stage IB or II cutaneous melanoma, aged over 16 years, and capacity to complete the study questionnaire. Eligible patients were also ascertained through the main private pathology services in Queensland, when pathologists included a standard note about the study on all relevant histopathology reports. The note informed treating doctors about the study and asked them to notify the pathology company if their patients should not be contacted. Patients for whom no objection was obtained within 2 weeks were sent information about the study with request for permission to release their details to study personnel who then invited the patients concerned to take part with signed consent. The study was approved by the Human Research Ethics Committees of the Metro South Hospital and Health Service and the QIMR Berghofer Medical Research Institute.

Data collection

All participants completed a standard self-administered questionnaire at baseline giving personal details, including sex, age, height, weight, highest level of education (less than year 12, technical college or diploma, university), smoking history (current, ex-smoker, never smoked), total lifetime number of painful sunburns (0-1, 2-5, >6) and skin type (always burn/never tan, burn then tan, tan only). Participants indicated amount of time spent outdoors on an average weekend (<5hours, 5-8hours) and whether their main occupations were indoors, both indoors and outdoors, or outdoors. Patients were asked to indicate frequency (never, less than 50%, more than 50%, all the time) of use of sun protection measures (including hats, sunscreen, long-sleeves, and sunglasses).

Positive histories of previous melanoma were confirmed by obtaining histology reports. Information about melanoma in a first degree relative (yes, no) was obtained and whether participants had been diagnosed previously with diabetes, cardiovascular disease or hypertension (yes, no). As well, use (yes, no), dosage (mg), frequency (daily, weekly etc.) and starting and stopping dates of statins, NSAIDs and corticosteroids in the preceding 5 years were recorded at baseline. History of medication use was updated (with addition of history of aspirin use) by questionnaire for all active participants in April 2016. For patients who were lost to follow-up in April 2016, information on aspirin use was obtained from GP referral letters to specialist melanoma units regarding their index melanoma.

Histological details of all primary melanomas were extracted from histopathology reports including thickness (mm) and presence of ulceration (yes, no) or mitosis (per mm^2 or per high power field) or regression (yes, no) as well as site of melanoma (head or neck, trunk, upper limb, lower limb). If the histopathology report did not specify ulceration was present, ulceration was classified as absent.

Statistical analysis

Based on their responses to the baseline questionnaire or to the follow-up medication survey, or on information provided in clinical referral letters, patients were categorised as regular users of statins, aspirin or other NSAIDs, or corticosteroids if they had taken 3+ tablets per week, every week, for any continuous 3-month period in the 2 years prior to their diagnosis. If regular medication commencement or cessation date was unknown or missing for a patient, these were categorised as 'missing'.

Body mass index (BMI) was calculated as weight (kg) / height (m)^2 and categorised as underweight/normal <25, overweight 25-30 or obese >30. We created a summary variable for use of sun-protection measures (sunscreen, protective clothing, hats and sunglasses). Subjects' adherence to sun-protection measures for >50% of time outdoors were categorised as nil, at least 1, 2-4 measures used. A combined anti-inflammatory variable was created based on regular use of either statins, aspirin or NSAIDs. Mitotic rate was categorised as <2, 2-6, >6 mm^2 prior to analysis based on the observed distribution of mitotic rate (when mitotic rate was reported per hpf (n=23), it was first converted to rate per mm^2). When mitotic rate was only qualitatively described in the pathology report (n=21) it was coded as missing.

Pearson's chi-squared tests were used to examine associations between tumor ulceration status and patient and tumor characteristics. Multivariable logistic regression analyses were conducted to assess the associations between inflammatory factors and presence of ulceration, controlling firstly for age and sex, and then for age, sex, melanoma thickness and mitotic rate.

Interactions between clinical, histological and inflammatory parameters with ulceration status were explored in exhaustive best-subset selection, lasso (Tibshirani, 2011) and stepwise regression model selection techniques. Variables with p<0.9 on univariate analysis were included in model building analyses and p<0.1 was used as the selection

criterion for the stepwise regression approach. Under the exhaustive selection method, models were ranked based on observed Akaike information criterion (AIC) values. Our lasso criteria chose the regularization parameter $\lambda = \rho^i$ where $\rho = 0.8$ and i is the ith step in the selection process as implemented in SAS 9.4. We used the area under the Receiver Operating Characteristic curve (AUC ROC) to assess discriminative ability of the selected models.

Due to the strong association between ulceration, mitosis and tumor thickness confirmed in model selection analyses, we also performed a subgroup analysis, nominated *a priori*, in patients whose melanomas were ≤2mm thick and ≤2/mm^2 mitotic rate in order to entirely eliminate confounding by thick or highly mitotic melanomas. For the purposes of this paper, we refer to this subgroup as melanomas with relatively low proliferation. Due to the small numbers of exposed cases, smoking and corticosteroid use were not included in the subgroup analyses, and use of either aspirin or NSAIDs or statins was analysed as a combined variable. To identify associations of pro-and anti-inflammatory factors and ulceration in those with relatively low proliferation melanomas, we conducted multivariable regression analyses adjusting for age and sex alone and for age, sex, BMI, diabetes and statin use. All statistical analyses were performed using SAS software version 9.4.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Table 1. Baseline characteristics of 787 patients with primary melanoma in relation to presence of melanoma ulceration

	Total (n=787) N (%)	Ulcerated (n=194) N (%)	Not Ulcerated (n=593) N (%)	Chi-square P-value
Patient factors				
Age (years)				
≥65	377 (48)	108 (56)	271 (45)	0.02
< 65	410 (52)	86 (44)	322 (55)	
Sex				
Male	447 (57)	125 (64)	322 (54)	0.01
Female	340 (43)	69 (36)	271 (46)	
Education				
High school or less	403 (51)	110 (57)	293 (50)	0.23
Trade/ Diploma	228 (29)	50 (26)	178 (30)	
University	155 (20)	34 (17)	121 (20)	
Occupation				
Indoors	274 (35)	62 (32)	212 (35)	0.01
Both	381 (48)	86 (44)	295 (50)	
Outdoors	132 (17)	46 (24)	86 (15)	
Weekend sun				
<4 hours	564 (73)	130 (70)	434 (74)	0.44
5-8 hours	209 (27)	57 (30)	152 (26)	
Lifetime sunburns				
Never/ Once	87 (11)	26 (13)	61 (10)	0.34
2-5	264 (33)	68 (25)	196 (33)	
>5	435 (56)	100 (52)	335 (57)	
Sun protection				
Never	125 (16)	27 (14)	98 (16)	0.67
Use 1 >50%	427 (54)	108 (56)	319 (54)	
Use 2-4 >50%	235 (30)	59 (30)	176 (30)	
Skin type				
Always burn	307 (39)	70 (36)	237 (40)	0.25
Burn then tan	398 (51)	99 (51)	299 (51)	
Tan only	78 (10)	25 (13)	53 (13)	
Previous melanoma				
No	623 (79)	160 (83)	463 (78)	0.19
Yes	164 (21)	34 (17)	130 (22)	
Family history				
No	565 (73)	140 (73)	425 (73)	0.88
Yes	213 (27)	51 (27)	163 (27)	
Primary melanoma factors				
Thickness (mm)				
<1	206 (26)	21 (11)	185 (31)	<0.0001
1.01-2.00	334 (43)	75 (39)	259 (44)	
2.01-4.00	176 (22)	58 (30)	118 (20)	
>4	69 (8)	40 (21)	29 (5)	
Mitosis (no./mm^2)				
<2	286 (37)	35 (18)	251 (44)	<0.0001
2-6	328 (43)	76 (40)	252 (44)	
>6	152 (20)	81 (42)	71 (12)	
Regression				
No	497 (63)	132 (68)	365 (62)	0.12
Yes	288 (37)	62 (32)	226 (38)	
Body site (location)				
Head/neck	167 (21)	44 (23)	123 (21)	0.35
Trunk	273 (35)	73 (38)	200 (34)	
Upper limb	165 (21)	32 (16)	133 (22)	
Lower limb	182 (23)	45 (23)	137 (23)	

For some variables, the summed total is less than the total number of patients because of missing values

Table 2. Factors associated with pro- and anti-inflammatory effects in relation to melanoma ulceration

	Total (n=787) N (%)	Ulceration (n=194) N (%)	No Ulceration (n=593) N (%)	Multivariate[1] OR (95% CI)	Multivariate[2] OR (95% CI)
Pro-inflammatory factors					
BMI					
<25	210 (29)	49 (27)	161 (29)	1.00	1.00
25-29.9	298 (41)	83 (46)	215 (39)	1.19 (0.78-1.77)	1.27 (0.80-2.01)
≥30	221 (30)	48 (27)	173 (32)	0.89 (0.56-1.40)	0.93 (0.56-1.53)
Smoking					
Nonsmoker	396 (53)	97 (52)	297 (53)	1.00	1.00
Current	59 (8)	15 (8)	44 (8)	1.11 (0.59-2.13)	1.09 (0.55-2.16)
Ex-smoker	295 (39)	74 (40)	220 (39)	0.99 (0.69-1.41)	0.97 (0.66-1.43)
Hypertension					
No	220 (67)	22 (45)	198 (31)	1.00	1.00
Yes	109 (33)	18 (55)	22 (69)	0.94 (0.65-1.35)	0.85 (0.57-1.27)
Diabetes					
No	686 (87)	166 (86)	517 (88)	1.00	1.00
Yes	101 (13)	28 (14)	73 (12)	1.06 (0.65-1.71)	1.11 (0.66- 1.88)
Heart disease					
No	684 (87)	162 (84)	520 (70)	1.00	1.00
Yes	103 (13)	32 (16)	70 (12)	1.23 (0.76-1.98)	1.29 (0.77- 2.18)
Anti-inflammatory factors					
Statins					
No	556 (72)	143 (75)	410 (71)	1.00	1.00
Yes	218 (28)	48 (25)	170 (29)	0.67 (0.45-0.99)	0.64 (0.42-0.98)
Aspirin					
No	560 (78)	137 (79)	420 (78)	1.00	1.00
Yes	155 (22)	36 (21)	119 (22)	0.76 (0.49-1.18)	0.78 (0.48-1.26)
NSAIDS					
No	658 (97)	156 (97)	500 (97)	1.00	1.00
Yes	21 (3)	5 (3)	16 (3)	1.04 (0.37-2.91)	0.79 (0.26-2.43)
Anti-inflammatory[3]					
No	489 (62)	125 (64)	361 (61)	1.00	1.00
Yes	298 (38)	69 (36)	229 (39)	0.72 (0.50-1.03)	0.68 (0.46-1.00)
Corticosteroid					
No	723 (93)	182 (94)	540 (93)	1.00	1.00
Yes	55 (7)	11 (6)	43 (7)	0.69 (0.35-1.38)	0.55 (0.25- 1.19)

1- Adjusted for age and sex

2- Adjusted for age, sex, thickness and mitosis

3- Regular use of either statins, aspirin or NSAIDS

For some variables, the summed total is less than the total number of patients because of missing values

Table 3. Pro-inflammatory factors associated with melanoma ulceration in a population subgroup (mitosis $\leq 2/mm^2$ and thickness $\leq 2mm$)

	Ulceration 40 (12) N (%)	No ulceration 289 (88) N (%)	OR (95% CI)[1]	OR (95% CI)[2]
Pro-inflammatory factors				
BMI				
<25	6 (16)	86 (31)	1.00	1.00
25-29.9	19 (50)	106 (39)	2.60 (0.99-6.84)	2.63 (0.99-6.96)
≥30	13 (34)	81 (30)	2.32 (0.84-6.41)	2.06 (0.70-6.02)
Hypertension				
No	22 (55)	198 (68)	1.00	1.00
Yes	18 (45)	91 (32)	1.91 (0.93-3.92)	1.91 (0.84-4.32)
Diabetes				
No	30 (75)	258 (89)	1.00	1.00
Yes	10 (25)	31 (11)	2.89 (1.26-6.60)	2.90 (1.07-7.90)
Heart disease				
No	33 (83)	257 (89)	1.00	1.00
Yes	7 (17)	32 (11)	1.70 (0.68-4.30)	1.10 (0.38-3.22)
Anti-inflammatory factors				
Statins				
No	29 (73)	207 (73)	1.00	1.00
Yes	11 (27)	78 (27)	0.97 (0.44-2.12)	0.68 (0.26-1.73)
Anti-inflammatory[3]				
No	25 (63)	185 (64)	1.00	1.00
Yes	15 (37)	104 (36)	1.04 (0.51-2.14)	0.85 (0.37-1.97)

1- Multivariable regression adjusted for age and sex

2- Multivariable regression adjusted for age, sex, BMI, diabetes and statin use

3- Regular use of either statins, aspirin or NSAIDS

For some variables, the summed total is less than the total number of patients because of missing values