Clinical significance of intra-nodal naevi in sentinel node biopsies for malignant melanoma

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Abstract

Background

Intra-nodal naevi (INN) identified during assessment of a sentinel lymph node for melanoma are not an uncommon finding. Little is known about their clinical significance. Patients with INN are treated as sentinel node biopsy (SNB) negative currently. Our aim was to assess the significance of INN in patients who undergo SNB for melanoma.

Methods

353 melanoma patients who underwent a SNB between November 1999 and June 2012 were retrospectively analysed from a prospectively collected database. The patients were divided into SNB negative, INN, isolated tumour cells (ITC) and SNB positive groups. Outcome measures of nodal recurrence, distal recurrence and survival were used to assess the differences between the groups.

Results

203 patients were SNB negative, 103 were positive of which 13 had ITC, 47 had INN (13%). Overall median follow up was 2.3 years (range 0.1 – 14.1 years). Our data demonstrated a statistically significant survival benefit for patients who had an INN compared to the SNB positive and ITC group. INN patients also had significantly better nodal and regional recurrence compared to SNB positive patients. There was no difference between INN and SNB negative patients.

Conclusion

We have clinically demonstrated that patients with INN on SNB can be adequately treated as SNB negative patients.

Keywords: Melanoma; metastatic melanoma; sentinel node biopsy; intra-nodal naevi; isolated tumour cells

Introduction

Sentinel node biopsy (SNB) is a well established and accepted staging tool for malignant melanoma (MM) patients. It involves a triple diagnostic technique, using lymphoscintography, blue dye and radio colloid with gamma probe detection. SNB allows upstaging of patients with isolated melanoma cells and micrometastasis which can permit early intervention with nodal basin clearance before potential progression into palpable stage III disease. However the benefits of early intervention for micrometasis have not been proven by clinical trial and await the results of the Multicenter selective lymphadenectomy trial II (MSLT-II).

The detection of micrometastases and isolated tumour cells within sentinel node biopsies has been improved by advances in immunohistochemistry techniques. INN (also referred to as nodal naevi and naevus cell aggregates) are not an uncommon finding in sentinel node biopsies, particularly in axillary nodes (1-3). They are less frequently found in lymphadenectomy specimens, most likely due to the more thorough sectioning techniques used in SNB. INN are often found as isolated clusters of normal-appearing melanocytes within the capsule, trabeculae, and rarely the parenchyma or lymphatic channels, of a lymph node (3,4). A histological example of an INN is shown in **Figure 1**. INN can be present in several malignancies including breast carcinoma, squamous cell carcinoma of the skin and most commonly melanoma, with the incidence of INN in all malignancies reported as 1-24% (2,5,6).

Two controversial hypotheses regarding their origin exist: I) there is regional embolic drainage of melanocytes from a naevus to a lymph node via the lymphatics (7,8); II)

embryological neural crest derived melanocytes are transported to lymph nodes during in-utero migration (7,9).

Histologically it can be difficult to differentiate INN from nodal metastases and a combination of immunohistochemistry, location and morphology must be used although this is not consistently reliable. This provides significant diagnostic challenges where false positive and negative SNB findings could lead to over or under treatment.

Evidence of the clinical significance of INN is limited with only one study evaluating the clinical outcome of patients with INN versus positive SNB (10). Several authors have noted a significant association between primary cutaneous melanoma and the presence of INN (2, 8, 10-12). Some authors even suggest an, as yet unproven, association between INN and melanoma of unknown primary (5).

Current British Association of Dermatology/British Association of Plastic, Reconstructive and Aesthetic Surgery (BAD/BAPRAS) guidelines do not recommend a specific treatment option for INN in SNB (13). MSLT-I classified INN as SNB negative however they did not analyse these patients as a specific subgroup (14). Current standard practice in the UK sees patients with INN treated as SNB negative.

The aim of this paper is to evaluate the clinical significance of INN in patients who undergo SNB for MM and to assess whether they should be classified as SNB negative.

Methods

This study was carried out at the Christie Hospital in Manchester, UK. Patients with malignant melanoma confirmed on excision of primary tumour who underwent a SNB

between November 1999 and June 2012 were retrospectively analysed from a prospectively collected database. Indication for SNB was a primary tumour Breslow thickness of 1-4mm, or patients with tumours less than 1mm with additional high risk factors including ulceration, high mitotic count, perineural spread, Clark level IV or greater. All patients undergoing SNB had clinical disease excluded through examination and staging CT.

SNB was carried out using a standard triple diagnostic technique with lymphoscintigraphy, blue dye and gamma probe assessment. Histopathological assessment of the lymph nodes were performed using a standardised method, as recommended by the European Organisation for Research and Treatment of Cancer (EORTC) (15). This involved a dedicated histopathology team using serial sectioning at 50 microns, H&E and S100 stains with additional immunohistochemistry staining as required. Positive SNB results were classified as ITC, metastases 0.1-2mm and metastases >2mm in line with previously published studies (16,17). All patients with positive SNB underwent completion lymphadenectomy, those that declined further surgery were excluded from the study.

Data was collected with regard to demographics, location of the primary, Breslow thickness, histology of the SNB, local and distant recurrence and survival. All follow-up data was added prospectively to the database.

The patients were divided into SNB positive, ITC on SNB, SNB negative and INN groups. Patients with ITC were chosen as a separate comparative group as they contain the lowest burden of metastasis within a positive sentinel lymph node. INN identified at completion lymphadenectomy were excluded from the study.

Outcome measures of nodal recurrence, distal recurrence and 5-year survival were used to assess the differences between the groups. Difference between INN patients and sentinel node positive, sentinel node negative and ITC patients were analysed. Recurrence and survival were calculated from the time of diagnosis of primary melanoma. Breslow thickness, ulceration, histological subtype and location of primary were all evaluated for an effect on survival. Clark level and mitotic rate were not included in the analysis due to insufficient data.

Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) Version 16 (IBM, USA). Estimated survival was calculated using Kaplan-Meier curves. Significance was calculated using log rank tests and chi squared tests for categorical data. A p-value of <0.05 was considered significant.

Results

Between November 1999 and June 2012, 353 patients with a median age of 53.5 years, underwent SNB for MM and were included in the study. Demographics of all included patients are summarised in **Table 1**.

Forty seven patients (13%), 16 male and 31 female, with a median age of 52.5, had INN detected within their SNB. Median Breslow thickness was 1.5mm (range 0.6-4mm).

203 patients (58%) were sentinel node negative. 103 patients were sentinel node positive (29%), of these 13 (4%) had isolated tumour cells, 63 (18%) had metastases 0.1-2mm, and 27 (8%) had metastases >2mm. A higher Breslow thickness was significantly associated with a positive sentinel node biopsy ($p<0.05$). There was no significant difference in any other demographic characteristic between any of the groups.

The presence of nodal naevi was more commonly associated with the female sex and upper limb primaries however these differences were not significant ($p>0.05$). INN were not significantly associated with any other demographic characteristic.

Breslow thickness, ulceration, histological subtype and location of primary were not significant for an effect on survival or recurrence in any of the groups.

Nodal Recurrence

Less than 1% of sentinel node negative patients and no INN or ITC patients developed nodal recurrence with no significant difference in recurrence between these groups ($p>0.05$). There was a significant difference in nodal recurrence between SNB positive and INN patients ($p<0.01$).

Distant recurrence

There was no significant difference in the frequency of distant recurrence between INN and SNB negative patients. There was a significant difference in distant recurrence between INN and SNB positive patients ($p<0.001$). There was also a significant difference between INN and ITC patients in distant recurrence ($p<0.05$) as illustrated in **Figure 2**.

Survival

The 5 year survival rate of the different groups were: SNB negative patients 97.5%; INN patients 100%; ITC patients 80.8%; Mets 0.1-2mm 75.6%; Mets >2mm 43.7%. The survival curves are illustrated in **Figure 2**. There was a significant difference between INN patients and SNB positive patients as a whole ($p<0.001$) as illustrated in **Figure 3** and with each positive group. There was also a significant difference in survival between positive patients with smaller, <2mm metastases and larger, >2mm

metastases ($p<0.05$). There was no significant difference in survival between INN and SNB negative patients.

Discussion

The prevalence of 13% of patients with intra nodal naevi in sentinel node biopsy in this study is in line with previously reported rates (2,5,6). No significant factors were to be associated with the presence of INN in this study. However there was a trend towards significance of upper limb primary tumours which is in keeping with previous studies which have found INN to be more commonly associated with axillary nodes (2,3,18) and lower limb tumours to be negative predictive factors (10). Previous studies have found an association with tumour thickness (12), which we did not observe.

The presence of INN can pose a significant histological challenge. Melanoma cells may demonstrate a nevoid pattern with a lack of clearly atypical cells making them difficult to differentiate from benign cells (19-22). Moreover, loss of expression in HMB-45 can occur in approximately 20% of melanoma metastases (23). INN are often identified by location in the trabeculae or lymph node capsule, however aggregates have been identified within parenchyma and nodal sinuses (4,24,25).

In this study there were no false negatives amongst INN patients with no patients upstaged following nodal recurrence. This suggests that the standardised EORTC method for differentiating INN from metastatic deposits is robust and accurate when conducted correctly (15).

INN are known to be more common in melanoma than in other primary tumours (2). Theories for this include: mechanical disruption by the primary tumour forcing benign naevi into lymphatic channels (2,4) or an increased body melanocyte-load increasing the initial risk of melanoma (26).

Evidence for the clinical significance of INN in melanoma is limited with only one previously reported case of lymph node melanoma arising from dysplastic naevus deposits (27). Nodal naevi have been shown to harbour the BRAF V600E mutation (28) however this is commonly seen in benign melanocytes with no associated link between this and malignant INN change. Some have hypothesised that INN may provide a clue to metastatic melanoma of unknown primary (5,27) however this is also unproven.

To date only one study has attempted to evaluate the significance of INN in SNB for melanoma (10). The authors found that patients with INN had significantly better 5-year survival compared with SNB positive patients with no difference in survival between INN and SNB negative patients. Our study is in keeping with this; however we also found that there is a significant difference in both local and distant recurrence between INN and SNB positive patients whereas there is no difference when compared to SNB negative patients. This should be expected given the difference in survival but these findings indicate that the underlying biology of the nodal naevi cells is benign, at least within the relatively short followup of this study.

Interestingly we did not find any INN associated with metastatic deposits in SNB samples, which may provide clues to the underlying tumour biology associated with the primary tumours in INN. The benign nature of INN is further highlighted by the significant difference in both survival and distant recurrence between patients with INN and those with the lowest tumour burden, isolated tumour cells. We believe these findings confirm that patients with INN can be appropriately treated as SNB negative patients with no need for further treatment.

Limitations of this study include the relatively short followup time which limits the accuracy of any long term conclusions drawn on the behaviour of intra nodal naevi patients. A further limitation is the relatively small population size of both INN and ITC patients which limits the accuracy of the conclusions drawn for these groups. Further genetic evaluation of intra nodal naevi cells may allow their behaviour and malignant/metastatic potential to be further elucidated.

Conflict of interest statement

None

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Tables

Characteristic	Value
Age (Years)	
Median	53.5 (18.5-86)
Sex n(%)	
Male	146 (41)
Female	207 (59)
Follow up (months)	

Median (Range)	27 (2-169)
Site of primary tumour	
Lower limb	127
Upper limb	100
Trunk	116
Head and neck	10
Histological subtype of primary tumour	
Nodular	97
Superficial spreading	121
Acral lentiginous	27
MM unknown histology	108
Sentinel node biopsy result	
Negative	203
Intranodal naevi	47
Isolated tumour cells	13
Metastasis 0.1-2mm	63
Metastasis >2mm	27
Breslow thickness of primary (mm)	
Median (Range)	1.8 (0.4-12)
Ulceration of primary	
Yes	127
No	180
Unknown	48

Table 1: Summary of demographic data for all patients undergoing sentinel node biopsy

Figure legends

Figure 1: Histology slide illustrating an INN within a lymph node capsule

Figure 2: Kaplan-Meier curve illustrating difference in distant recurrence between patients with negative sentinel node biopsy, intra nodal naevi and isolated tumour cells

Figure 3: Kaplan-Meier curve illustrating difference in survival between patients with negative sentinel node biopsy, INN, ITC, metastases 0.1-2mm, and metastases >2mm

Figure 4: Kaplan-Meier curve illustrating difference in survival between INN and SNB positive patients

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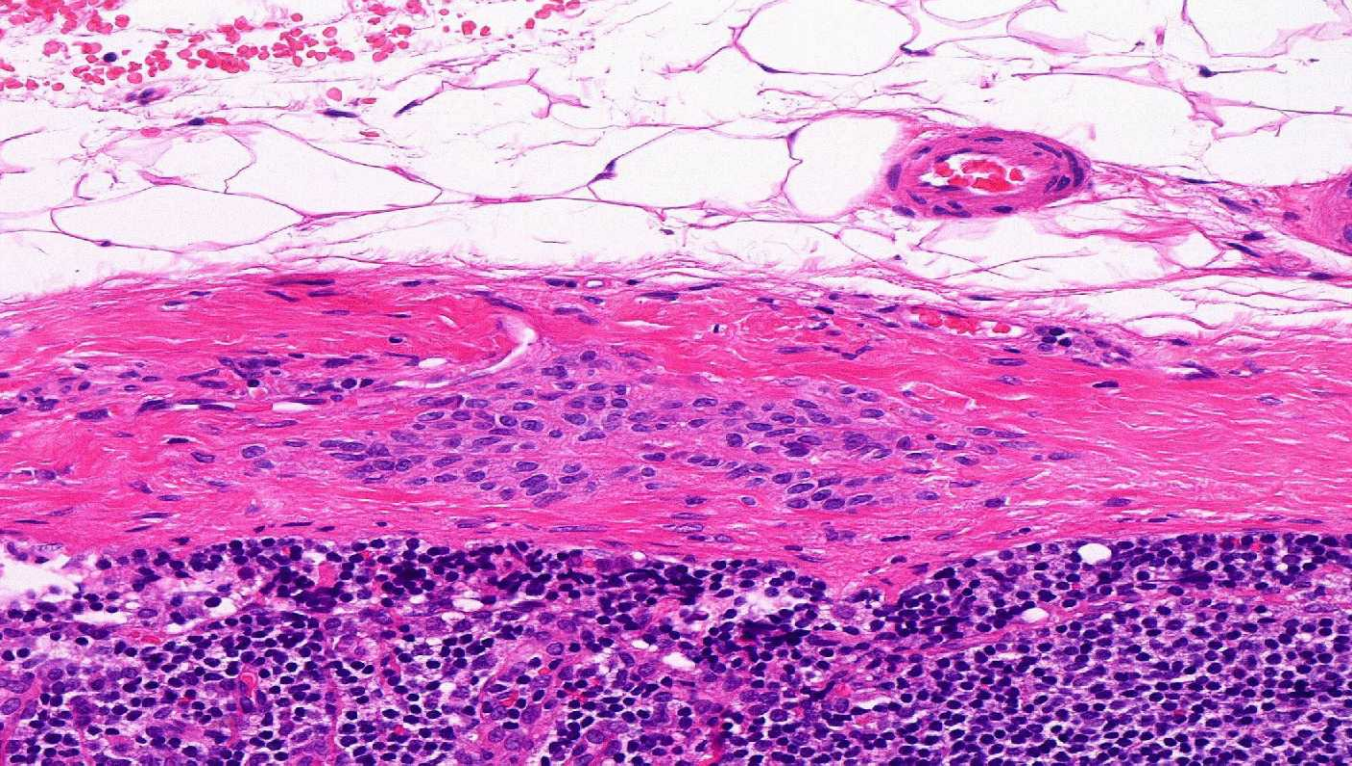

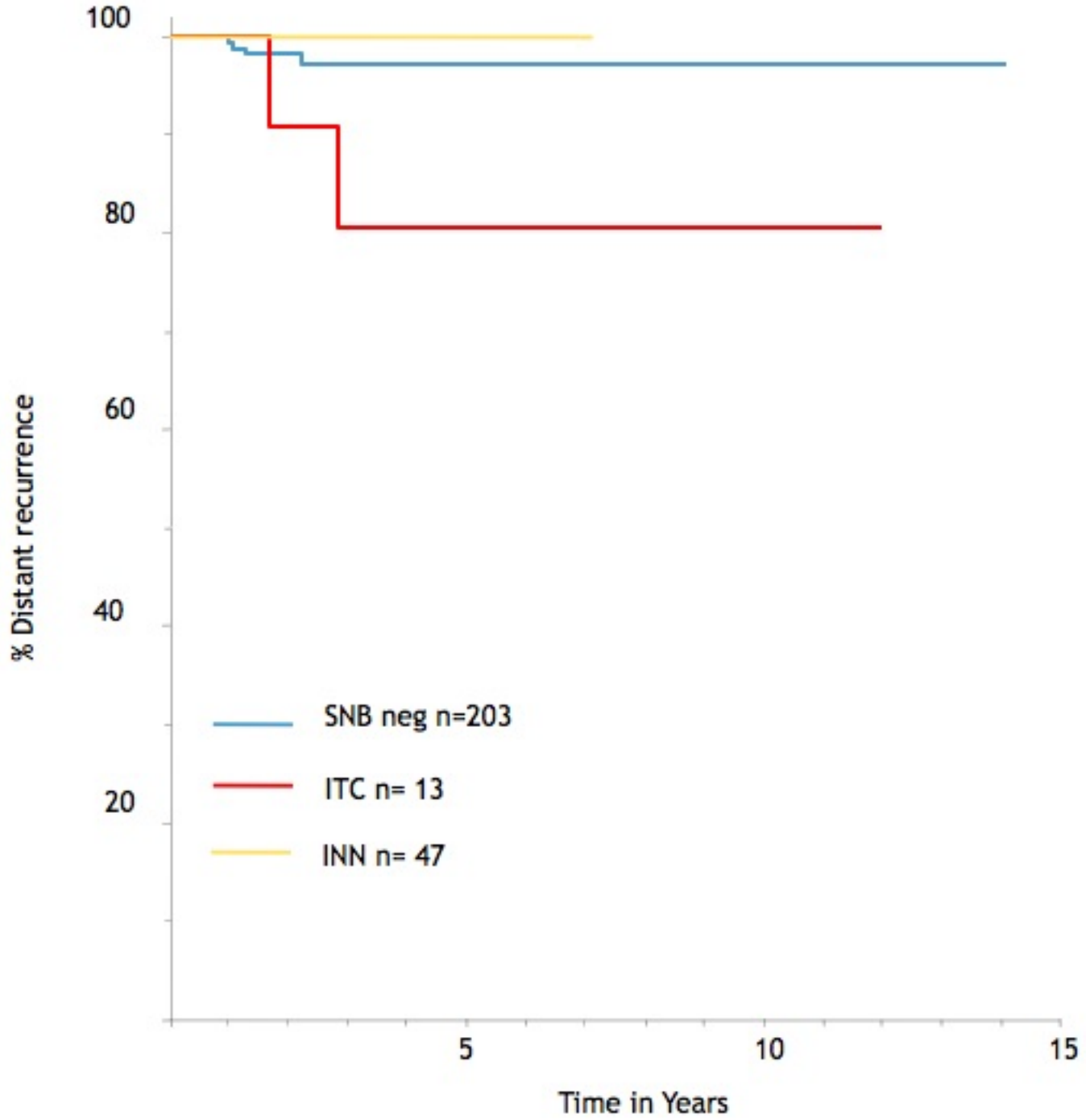
100
80
60
40
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% Distant recurrence
SNB neg n=203
ITC n= 13
INN n= 47
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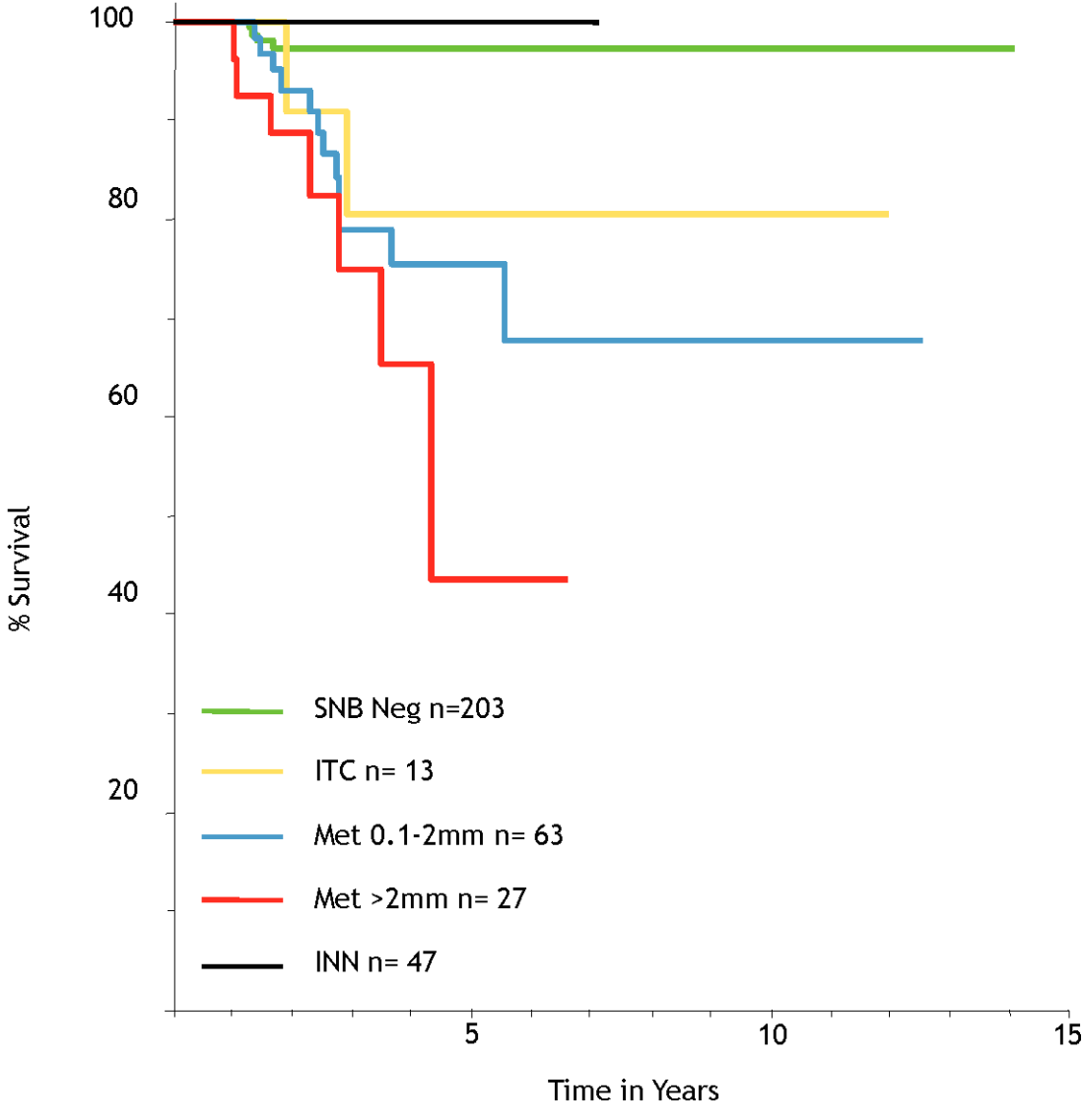
Time in Years


100
80
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% Survival
SNB Neg n=203
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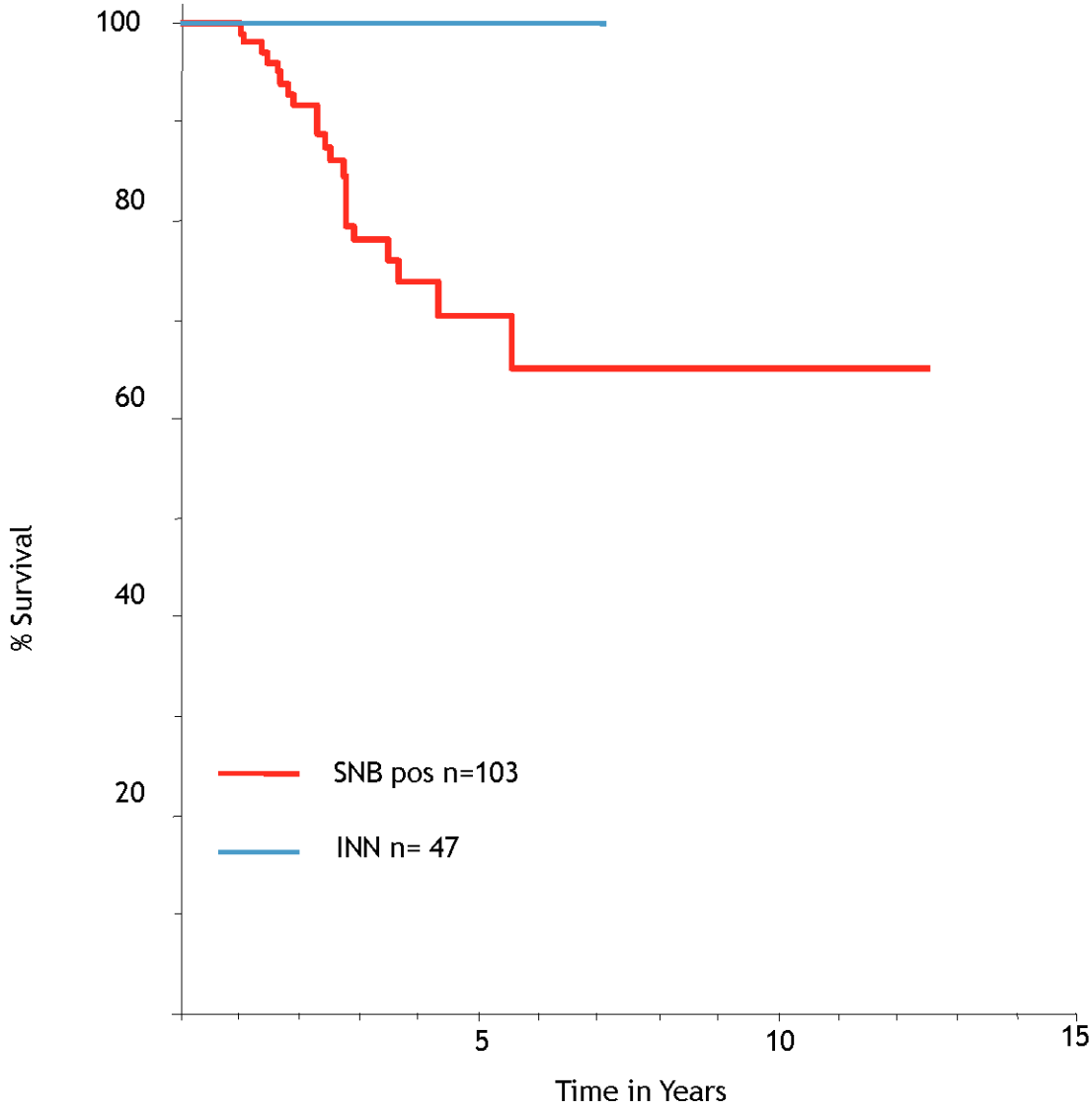
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% Survival
SNB pos n=103
INN n= 47
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Time in Years