ETHICAL APPROVAL

Ethical approval was granted by the QIMR Berghofer Medical Research Institute HREC approval P2200.

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REFERENCES

1. Wolff T, Tai E, Miller T. Screening for skin cancer: an update of the evidence for the U.S. Preventive Services Task Force. *Ann. Intern. Med.* 2009; **150**: 194–8.
2. Guther S, Ramrath K, Dyall-Smith D *et al*. Development of a targeted risk-group model for skin cancer screening based on more than 100,000 total skin examinations. *J. Eur. Acad. Dermatol. Venereol.* 2012; **26**: 86–94.
3. Verkouteren JAC, Smedinga H, Steyerberg EW *et al*. Predicting the Risk of a Second Basal Cell Carcinoma. *J. Invest. Dermatol.* 2015; **135**: 2649–56.
4. Whiteman DC, Thompson BS, Thrift AP*et al*., Study QS. A Model to Predict the Risk of Keratinocyte Carcinomas. *J. Invest. Dermatol.* 2016; **136**: 1247–54.
5. Olsen CM, Green AC, Neale RE *et al*. Cohort profile: the QSkin Sun and Health Study. *Int. J. Epidemiol.* 2012; **41**: 929–i.
6. Thompson BS, Olsen CM, Subramaniam P *et al*. Medicare claims data reliably identify treatments for basal cell carcinoma and squamous cell carcinoma: a prospective cohort study. *Aust. N. Z. J. Public Health.* 2016; **40**: 154–8.
7. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin. Chem.* 1993; **39**: 561–77.

Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Table S1. Medical Benefits Schedule (MBS) item numbers for the surgical treatment of first primary keratinocyte cancer.

Table S2. Acceptability of using the risk stratification tool in the Skin Clinic Validation Cohort (*n* = 96).

Research Letter

Dear Editor,

Appropriate indication and procedure for random skin biopsy in the diagnosis of intravascular large B-cell lymphoma

Intravascular large B-cell lymphoma (IVLBCL) is a very rare subtype of extranodal malignant B-cell lymphoma.[1,2] A useful method to diagnose IVLBCL is random skin biopsy, taken from healthy-appearing skin.[3] However, no consensus exists regarding random skin biopsy methods, and there is insufficient data regarding its accuracy.[4] Thus, we aimed to determine potential correlations between positive diagnoses and biopsy techniques, serum lactate dehydrogenase (LDH) levels, as well as serum-soluble interleukin 2 receptor (sIL-2R) levels.

The study retrospectively reviewed 69 patients (43 males, average age: 65.6 ± 6.3 years, range: 21–87 years) who visited our department between April 2013 and March 2018 for random skin biopsy.

Clinically normal samples were obtained from at least three separate areas under local anaesthesia: incisional excision in 45 patients, punch biopsy in 24 cases. Biopsy specimens were stained with anti-CD20 and CD79a antibodies.

A potential association between positive diagnoses and biopsy techniques was evaluated using Fisher Exact Test. Plotting the data showed that neither serum LDH nor sIL-2R levels were normally distributed. Therefore, between-group differences were determined using the Mann–Whitney U test. All analyses were conducted in SPSS (IBM, Japan). Significance was set at $P < 0.05$.

We diagnosed 10 patients with IVLBCL; the remaining 59 tested negative. One out of 10 IVLBCL-positive cases was detected using punch biopsy, and incisional biopsy detected the rest. Fisher exact test did not detect a significant difference between biopsy techniques.

Patients with IVLBCL had significantly higher ($P < 0.01$) serum LDH values (median = 639 U/L, range: 319–2962 U/L) than patients without IVLBCL (median = 208 U/L, range: 112–8408 U/L). Serum sIL-2R levels were highly variable, but again, we observed a
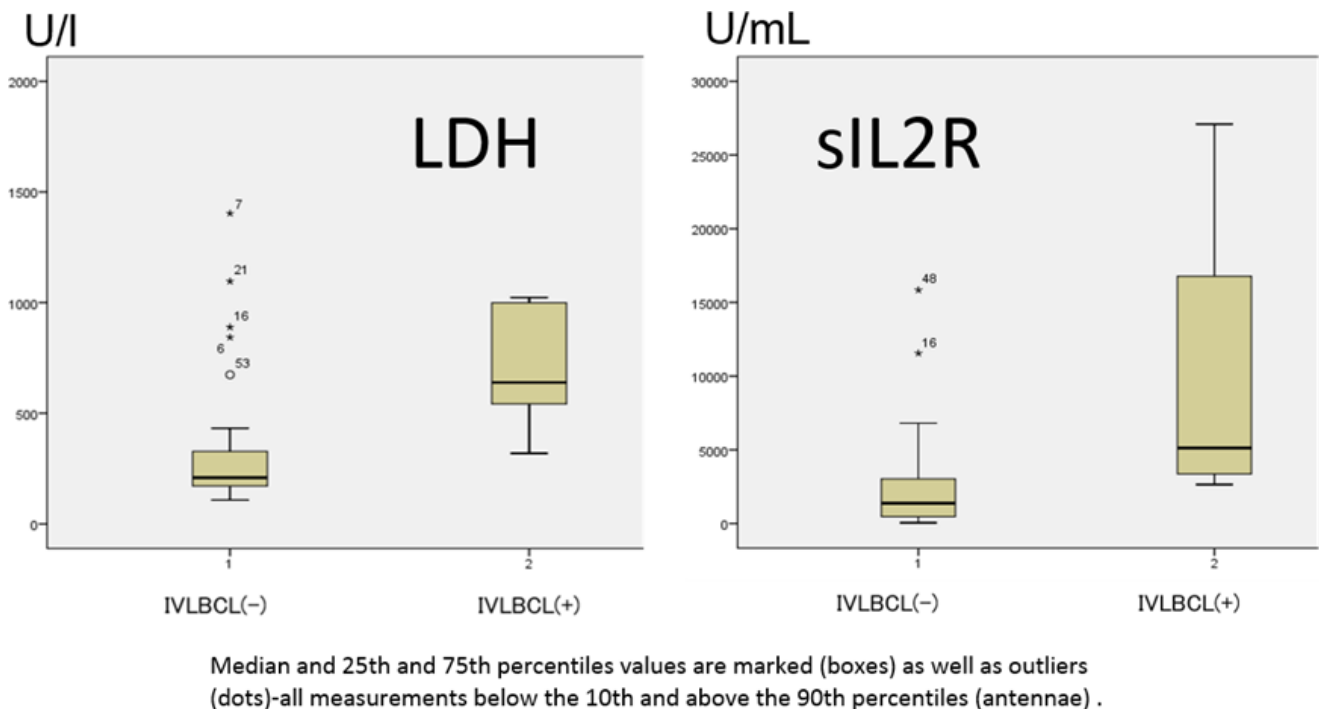
Figure 1 Box plot graphs of serum LDH levels and sIL-2R levels of IVLBCL negative and positive patients.

clear difference ($P < 0.01$) between patients with (median = 5120 U/mL, range 2646-27 093 U/mL) and without IVLBCL (median = 1373 U/mL, range: <50-15 833 U/mL) (Fig. 1).

We did not find significant differences in diagnostic accuracy among biopsy techniques, probably due to the small sample size. However, previous reports recommended against using punch biopsy as less tissue is available and results may not be representative.[4] Incisional biopsy from more than three separate areas, on the other hand, provides more fat tissue and capillaries, and is recommended to avoid false negatives.

Random skin biopsies are usually performed by dermatologists at our hospital. The procedure was mostly used for investigating unknown fevers, pancytopenia and cerebral infarction. For patients with particularly poor health or haematological disorders, we sometimes hesitated to perform incisional random skin biopsies due to possible postoperative haematoma in fat tissues. A previous study indicated five factors (low O_2 saturation, platelet count, serum LDH, serum sIL2R and unexplained fever) that helped to determine the indication for random skin biopsy in patients suspected of having IVLBCL.[2,5] In this study, we focused on serum LDH and sIL-2R because high fever, low O_2 saturation and low platelet count have low specificity, being often due to other complications.

In order to avoid false negatives in cases with serum sIL-2R >2000 U/mL, we recommend incisional biopsy from at least three separate sites. Because in this study,

serum sIL-2R levels were over 2000 U/mL for all IVLBCL-positive patients, whereas only 18 out of 59 IVLBCL negative patients were over 2000 U/mL. Conversely, when patients have sIL-2R <500 U/mL and normal serum LDH levels, we do not recommend random skin biopsy because these patients had no lymphoproliferative disorders in our study. Moreover, in cases where patients experience bleeding and coagulation abnormalities, random skin biopsy should be postponed to prevent unexpected serious bleeding.

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REFERENCES

1. Arai T, Kato Y, Funaki M *et al.* Three cases of intravascular large B-cell lymphoma detected in a biopsy of skin lesions. *Dermatology* 2016; **232**: 185–8.
2. Matsue K, Abe Y, Kitadate A *et al.* Sensitivity and specificity of incisional random skin biopsy for diagnosis of intravascular large B-cell lymphoma. *Blood* 2019; **133**: 1257–9.
3. Asada N, Odawara J, Kimura S *et al.* Use of random skin biopsy for diagnosis of intravascular large B-cell lymphoma. *Mayo Clin. Proc.* 2007; **82**: 1525–7.
4. Enzan N, Kitadate A, Tanaka A *et al.* Incisional random skin biopsy, not punch biopsy, is an appropriate method for

diagnosis of intravascular large B-cell lymphoma: a clinico-pathological study of 25 patients. *Br. J. Dermatol.* 2019; **181**: 200–1.

5. Matsue K, Asada N, Takeuchi M *et al*. A clinicopathological study of 13 cases of intravascular lymphoma: experience in a single institution over a 9-yr period. *Eur. J. Haematol.* 2008; **80**: 236–44.

Research Letter

Dear Editor

Inter- and intra-patient heterogeneity of PD-L1 expression in metastatic melanomas: A retrospective study

Immunotherapy with PD-1/PD-L1 axis blockers by inhibiting the binding of PD-1 with its ligands has become an important strategy in advanced melanoma. There is an objective response in 50% of patients with melanoma metastases, and the expression of PD-L1 is associated with a better outcome, but as a sole biomarker, it can be unreliable. Patients presenting PD-L1-negative metastatic melanomas can also respond to PD-1/PD-L1 axis blockers.[1] B lymphocytes, identified by the presence of CD20 in their membrane, began to be studied more recently in the context of melanomas as a potential biomarker in the monitoring and prognosis of patients.[2]

The objective of this retrospective cohort study was to analyse the expression of PD-L1 and CD20 in melanoma metastases, according to site, clinical and histopathological data, and survival. We assessed 50 metastatic melanomas in 46 patients. PD-L1 IHC was performed,[3] utilising E1L3N (R), clone (Cell Signaling Technology – Fig. 1a). We graduated the percentage of PD-L1 in melanoma metastases as negative for cases < 5% of tumour cells, and positive in three subcategories: 5–20%, 21–60%, and >60% of tumours cells expressing PD-L1. For the CD20 study, a similar immunohistochemistry technique was utilised using the clone L26 and the diaminobenzidine chromogen (both from Ventana, AZ, USA, Fig. 1b). CD20-positive cases were subclassified as intratumoral and/or peritumoral. To be classified as positive, CD20 lymphocytes should be intratumoral (within the melanoma bulk) and/or peritumoral (around the melanoma bulk, in close contact with its tumour cells). In the study of lymph nodes, cases in which the only location of the CD20 was in follicular centres (their natural location) were considered negative.

Table 1 correlates the histopathological data of metastatic and primary melanomas with the expression of PD-L1 in metastases. Of the 50 cases analysed, 22 (43%) were positive with expression between 5 and 60% of the cells. The age of the individuals ranged from 29 to 96 years (mean = 62 years). The majority were over 50 years old (37/46). The most frequent skin phototypes were II and III. We did not find a statistical difference between the expression of PD-L1 in metastases and the variables 'gender', 'age' or 'phototype'. Patient survival was not associated with the presence of PD-L1. Previous studies assessing survival and PD-L1 expression are contradictory: some relate better prognosis and longer disease-free time,[4,5] and others, a worse prognosis and less relapse-free time.[6,7] We
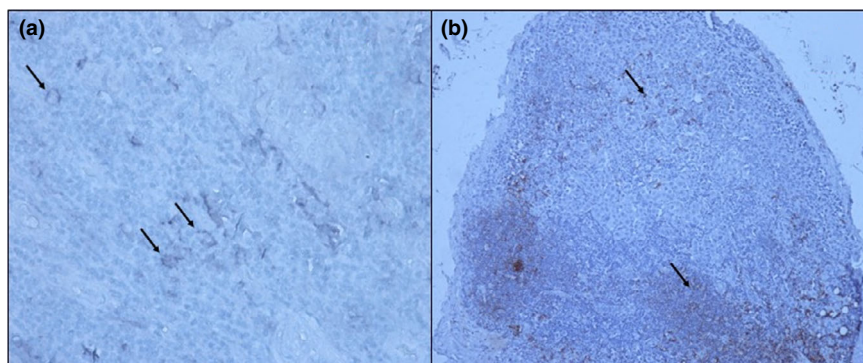

Figure 1 Immunohistochemistry of PD-L1 in metastatic melanomas using DAB/ nickel stain with Giemsa counterstaining (×200). (a) PD-L1-positive cells in lymph node and (b) negative PD-L1 central nervous system. (a) PD-L1-positive melanoma cells (arrows) of a metastatic melanoma in the lymph node. (b) note there is brown material (melanin) inside and among melanoma cells in the central nervous system, but the cells are PD-L1 negative.