The diagnostic and prognostic value of five blood markers in cutaneous T-cell lymphomas: a validation cohort

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INTRODUCTION

Clinical and laboratory diagnostics of mycosis fungoides (MF), the most common cutaneous lymphoma, is challenging. Our previous work described 4 promising blood markers of Sézary syndrome (SS): **T-plastin**, **Twist**, **NKp46** and **KIR3DL2** (Michel *et al.* Blood 2013). **Tox** has been shown to be an additional marker for MF and SS (Zhang *et al.* JID 2013).

AIM

The aim of the present study was to validate this combination of blood-derived biomarkers in a validation cohort of SS, erythrodermic and earlier MF, for improving diagnosis and predicting prognosis.

METHODS

Patients with a confirmed diagnosis of MF (n=221) or SS (n=156) and patients with other skin diseases (n=57) were consecutively included between 01/2011 and 12/2015. After written informed consent, blood samples and clinical data were collected including age, sex, diagnosis with stage, LDH, CRP, CD4/CD8 ratio, counts of leucocytes, counts of lymphocytes, counts of eosinophils, Sézary cell counts, natural killer (NK) cell counts, CD7⁺ cell percentage, CD26⁺ cell percentage.

Peripheral blood mononuclear cells (PBMC) were isolated by density gradient (Ficoll). CD4⁺ T-cells were purified by using positive magnetic cell sorting. Quantification of mRNA expression profiling was established on total PBMC and CD4⁺ T-cells by use of SYBR Green PCR Core (Thermo Fisher Scientific Inc) with specific primer pairs (*available on request*). Measures of gene expression were normalized with a house-keeping gene ($\beta 2M$).

Data were analysed descriptively (means, medians and standard deviations). To compare groups, independent samples t-test and Welch's t-test were used with an alpha 0.05 (two-tailed). To test the convergent and divergent validity Pearson correlation coefficients were calculated. Correlations with loss of CD7 and CD26 expression can be regarded as criterion validity, because these are the currently recommended parameters for the diagnosis of SS. To investigate the criterion validity and reliability, Receiver Operating Characteristics (ROC) curves were analyzed, areas under the curves (AUC) were calculated with thresholds of 95% specificity for each marker. Statistical analysis was performed using R statics.

References

Michel L *et al.* Use of PLS3, Twist, CD158k/KIR3DL2, and NKp46 gene expression combination for reliable Sézary syndrome diagnosis. 2013 Blood
Zhang Y *et al.* Molecular markers of early-stage mycosis fungoides. 2012 J Invest Dermatol.

RESULTS

A total of 156 patients with SS, 221 patients with MF and 57 patients with inflammatory dermatoses were included and compared. The expression of T-plastin, Twist, KIR3DL2 and Tox were statistically significant in **SS** and **erythrodermic MF** compared the other groups ($p \leq 0.001$ and, $p \leq 0.05$, respectively). The combination of T-plastin and Twist for the diagnosis of MF or SS had **95.2% specificity** and **84% sensitivity**. The addition of KIR3DL2 into the diagnosis model increase its **sensitivity to 94%** but decreased the specificity to 90%. Patients expressing KIR3DL2 had significantly lower time to relapse compared to the other groups : **~prognostic marker**

Patient characteristics

Diagnosis	n	female/male (%)	Median age at diagnosis (range)	Median CD4+/CD8+ ratio (range)	Median % of CD4 ⁺ CD7 ⁺ (range)	Median % of CD4 ⁺ CD26 ⁺ (range)
SS	156	46.4	66.9 (19.9-87.5)	12.51 (1.04-183.55)	63 (0.4-99.4)	58 (0.6-98)
eMF	36	38.2	63.7 (23.5-92.2)	46.24 (6.7-85.79)	26 (12-40)	42.5 (28-57)
MF	185	28.7	53.7 (17.4-95.1)	3.88 (0.93-49.78)	10 (4.3-68.6)	28.75 (8.5-83)
eBID	30	25	60.3 (25.8-88.8)	3.01 (2.56-14.72)	7 (4-7.4)	14 (13.6-24)
BID	27	23.1	60.5 (21.7-88.1)	4.1 (1.94-9.49)	49.95 (28-59.9)	55.6 (51.6-60.2)

SD: standard deviation; SS: Sézary syndrome; eMF: erythrodermic mycosis fungoides; MF: mycosis fungoides; eBID: erythrodermic benign inflammatory dermatosis; BID: benign inflammatory dermatosis

Correlation of the markers with gold standards

Pearson's correlation coefficients displayed as circles. Red and/or greater diameter corresponding to 1, blue and/or greater diameter corresponding to -1.

Receiver Operating Curves of the markers

Receiver Operating Characteristics (ROC) curves displaying the ability of the markers to distinguish between erythrodermic MF and SS

Expression of the markers among disease groups

SS: Sézary syndrome; eMF: erythrodermic mycosis fungoides; MF: mycosis fungoides; eBID: erythrodermic benign inflammatory dermatosis; BID: benign inflammatory dermatosis; CTCL: cutaneous T-cell-lymphoma; Welch's t-test: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$

Prognostic value of KIR3DL2

CONCLUSION

In conclusion, Twist, T-plastin and KIR3DL2 have high validity to distinguish MF and SS from other skin diseases. KIR3DL2 may predict the prognosis. These data bring new horizons to investigate the validity of these markers and their accuracy in clinical use, especially for KIR3DL2.