Morphological, immunophenotypical and molecular data analysis of peripheral blood samples in a series of patients with B- and T-cell primary cutaneous lymphomas

Maria Estela Martínez-Escala[1], Fernando Gallardo[1], Beatriz Bellosillo[3,4], Ramon M. Pujol[1], Eduardo Rozas[1], Mar García[4], Ester Moragon[3,4], Rosa Navarro[2,4], Sergi Serrano[4], Lourdes Florensa[2,4], Anna Ferrer[2,4]

[1]Department of Dermatology, [2]Hematologic Cytology Laboratory, [3]Molecular Biology Laboratory, [4]Department of Pathology. Hospital del Mar, Parc de Salut Mar, Barcelona, Spain

Background

Peripheral blood (PB) involvement by malignant lymphoid cells in primary cutaneous lymphomas (PCL) may have prognostic implications and recent staging procedures recommend a PB assessment in patients with mycosis fungoides (MF). Nevertheless, the routine practical usefulness of PB examination in the different subtypes of PCL has not been fully demonstrated. In this study the morphological, immunophenotypical and molecular data from PB samples in a large series of patients with PCL were evaluated.

Patients and methods

Patient samples (n=107). Eighty-eight consecutive patients with different subtypes of PCL followed at the Department of Dermatology, Hospital del Mar, were included in the study. Fifty-six patients corresponded to primary cutaneous T-cell lymphomas (CTCL): 37 MF, 6 Sézary syndrome (SS), 7 lymphomatoid papulosis (LyP), 3 CD30+ CTCL and 3 non-MF/non-CD30+ CTCL. Thirty-two patients presented primary cutaneous B-cell lymphomas (CBCL) distributed as follows: 22 marginal zone CBCL (MZL), 4 follicle centre cell CBCL (FCL) and 6 primary cutaneous large B-cell lymphoma, leg type (LCBCL, leg type) (Figure 1,2). Control PB samples (n=29) from 6 patients suffering from reactive lymphoid hyperplasia (RLH), 4 parapsoriasis, and 19 inflammatory dermatoses (ID) were also evaluated.

PB study protocol included cytomorphology exam and flow cytometry analysis. Four-color flow cytometry study using FacsCanto II Flow Cytometer (Becton Dickinson, San Jose, CA) was performed. Flow cytometric immunophenotyping included a complete panel of monoclonal antibodies specific for B- and T-cell (B-cell antibodies: CD19, CD20, CD22, CD79, kappa and lambda; T-cell antibodies: CD2, CD3, CD5, CD7, CD4, CD8, CD26, CD28, CCR7, TCR $\alpha\beta$, TCR$\gamma\delta$; Non-specific antibodies: CD45, CD16, CD56, CD57, CD11c, CD25, CD103).

B- and T-cell clonality assessment on skin and PB samples was performed by PCR according to BIOMED2 protocols and fluorescent fragment analysis (GeneScan 400 ROX, Applied Biosystems, Foster City, CA). TCR gene rearrangements included amplification of γ, β and δ subunits. A combined full approach of FR1, FR2 and FR3 primers for complete VDJ gene rearrangements of the Ig heavy chain, as well as DJ incomplete rearrangements, complete VJ rearrangements of the kappa light chain and rearrangements of the kappa deleting element were used in CBCL samples.

Figure 1: Primary cutaneous T-Cell lymphomas

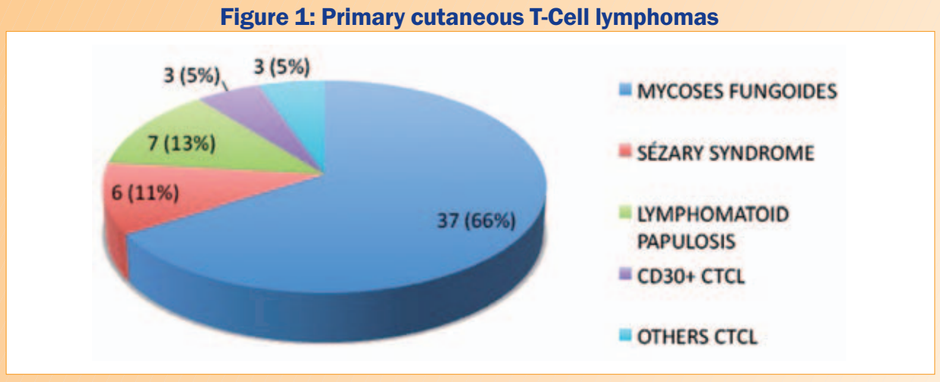
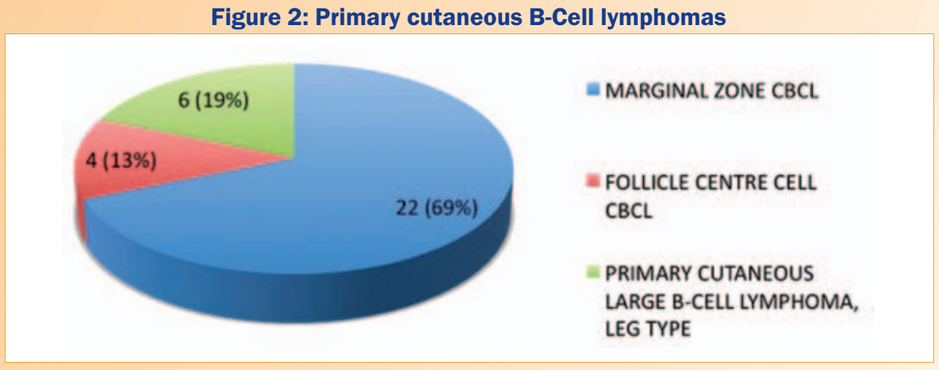


Figure 2: Primary cutaneous B-Cell lymphomas



Results and discussion

MF/SS group (n=37): Abnormal cytomorphology, corresponding to large granular lymphocytes was detected in three patients (9%) with plaque-stage MF, a feature considered to be non-specific. An abnormal immunophenotype was detected in five MF patients, (four patients with a double CD4-/CD8- lymphocyte population, and one patient with a CD4+/CD8+ phenotype). Two patients with CD4-/CD8- phenotypes showed PB/skin clonal concordance. A CD8+/CD57+ reactive immunophenotype was detected in three patients. Monoclonal T-cell dominance was detected in 22 PB samples (63%) from MF patients (Table 1). However, skin and PB clonal concordance was observed in only 3 cases, two MF stage IA and one MF stage IB. This latter patient presented a clinical evolution to SS (Table 2). All SS samples demonstrated abnormalities in cytomorphology, immunophenotype and molecular data. A lack of expression of CD7 and CD26 antigens and a low expression of CD2, CD3 and CD5 was commonly observed in SS patients.

Table 1

PRIMARY CUTANEOUS T-CELL LYMPHOMAS (N=56)			
PERIPHERAL BLOOD	MORPHOLOGY*	IMMUNOPHENOTYPE**	MOLECULAR***
MYCOSIS FUNGOIDES (N=37)	9% (3/33)	23.5% (8/34)	63% (22/35)
SÉZARY SINDROME (N=6)	100%	100%	100%
LYMPHOMATOID PAPULOSIS (N=7)	25% (1/4)	25% (1/4)	0% (0/7)
CD30+ CTCL (N=3)	0% (0/2)	0% (0/2)	100% (2/2)
OTHERS CTCL (N=3)	0% (0/3)	0% (0/2)	33% (1/3)

Table 2

MYCOSIS FUNGOIDES WITH SKIN AND PB CLONAL CONCORDANCE					
CASE	STAGE	MORPHOLOGY	IMMUNOPHENOTYPE	MOLECULAR	FOLLOW UP
1	IB	NORMAL	NORMAL	γ and β	Developed SS
2	IA	NORMAL	CD4-CD8-/+w[a]	γ	=
3	IA	LGL[b] 27%	CD4-CD8-/+w[a]	γ	=

[a]w: weak / [b]LGL: large granular lymphocytes (Normal <10%)

*Nº cases with abnormal cytomorphology / Nº cases that cytomorphology exam of PB sample were performed. **Nº cases with abnormal immunophenotype / Nº cases that flow cytometry analysis of PB sample were performed.
***Nº cases with monoclonal dominance / Nº cases that B- or T-cell clonality assessment on PB sample were performed.

CD30+ lymphoproliferative group (n=10): No clonal dominances were detected in any of the patients with LyP. One patient with CD30+ CTCL presented a monoclonal peak in PB, which was different from the observed dominant skin clone (Table 1).

Other CTCL groups (n=3): No PB abnormalities were detected in all except one patient included in this group. This patient presented a small to medium cell pleomorphic CD4+ CTCL, and a T-cell clone was detected which was no concordant with the observed skin one (Table 1).

T-cell control group (n=23): A T-cell clone was detected in 5/19 ID patient samples. No skin clonal dominance was detected in this group. These observations were considered to correspond to aged-related clonal proliferations (as all patients were older than 70 years) (Table 3).

CBCL group (n=32): The cytomorphology, immunophenotpe and molecular data of PB samples from CBCL group disclosed a B-cell clone in two patients with MZL not concordant with the skin results. One of them developed a systemic B-cell lymphoma after a 3 years follow-up (Table 4).

Table 3

		MORPHOLOGY*	IMMUNOPHENOTYPE**	MOLECULAR***
T-CELL CONTROLS	ERYTHRODERMAS (N=4)	1/3	1/3	1/3
	PRURITUS SINE MATERIA (N=2)	1/2	2/2	2/2
	DRUG ERUPTIONS (N=4)	0/4	0/4	1/3
	PITYRIASIS LICHENOIDES (N=2)	0/1	0/1	0/2
	OTHERS (N=7)	0/7	0/7	1/5
	PARAPSORIASIS (N=4)	0/2	0/2	0/4
B-CELL CONTROLS	REACTIVE LYMPHOID HYPERPLASIAS (N=6)	0/6	0/6	0/6

Table 4

PRIMARY CUTANEOUS B-CELL LYMPHOMAS (N=32)			
PERIPHERAL BLOOD	MORPHOLOGY*	IMMUNOPHENOTYPE**	MOLECULAR***
MARGINAL ZONE LYMPHOMA (N=22)	0% (0/22)	0% (0/22)	9% (2/22)
FOLLICLE CENTRE CELL LYMPHOMA (N=4)	0% (0/4)	0% (0/4)	0% (0/4)
LEG TYPE LYMPHOMA (N=6)	0% (2/2)	0% (2/2)	0% (0/6)

Conclusions

The detection of an abnormal immunophenotype and/or clonal dominance in PB seems to be a common feature in CTCL patients, particularly in MF, and can be also present in early stages of the disease. Nevertheless in our series clonal concordance between skin and PB was only demonstrated in a limited number of cases. The high detection rates of a clonal population on peripheral blood from CTCL patients could be explained as a malignant clonal heterogeneity, as well as the presence of reactive antitumor clones or age-related expanding cells. In order to avoid an overestimation of non-malignant clones in PB samples, some authors have stressed the importance to perform multiple PCR runs (analyzing different samples) when non-concordant PB-skin clones are detected.

In our series, a high rate of T-cell clonal proliferations in PB samples from CTCL patients was detected, but a clear-cut prognostic value of this finding could not be demonstrated. A close monitoring for those patients harbouring a skin/PB clonal concordance seems advisable. Conversely, our results show that PB assessment seems to have limited value in the work-up staging of patients with CBCL.

References

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Hospital del Mar — Parc de Salut MAR Barcelona