# Diagnostic and prognostic value of genotypic analysis in cutaneous lymphoproliferative processes using the standardized BIOMED-2 polymerase chain reaction protocols

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## Introduction & Objectives

The standardized BIOMED-2 polymerase chain reaction (PCR) protocols are widely used for detection of clonal populations of T- and B-cells and represent an important tool in the diagnosis of cutaneous lymphomas.[1-4] The purpose of this study was to assess the diagnostic and prognostic value of the genotyping results obtained by these techniques in daily clinical practice.

## Material & Methods

222 lesional skin biopsies were retrospectively reviewed from 93 patients listed in the Hospital del Mar Registry of Cutaneous Lymphoma and diagnosed according to the 2018 update WHO-EORTC classification[5] as: 71 cutaneous T-cell lymphomas (CTCLs) and 22 cutaneous B-cell lymphomas (CBCLs) (Table 1). 232 biopsy specimens from 168 patients with benign cutaneous lymphoid infiltrates were used as controls (Table 2). From all patients formalin fixed and paraffin embedded tissue was studied and in 123 cases extracutaneous samples (mostly peripheral blood) were also analyzed. T-cell receptor (TCR) and immunoglobulin (IG) rearrangements were assessed using the standardized BIOMED-2 PCR protocols.[1] Median follow-up was 5 and 4 years in cutaneous lymphomas and control subjects, respectively.

**Table 1. Characteristics of the primary cutaneous lymphomas cases**

| WHO-EORTC Classification 2018 | | N | Gender (M/F) | Age at diagnosis (mean in years) | Skin biopsies (sequential biopsies) | Blood samples | Follow-up (mean in years) |
|---|---|---|---|---|---|---|---|
| CTCLs | Early-stage MF (stages IA-IIA) | 35 | 24/11 | 54.5 | 96 (72) | 47 | 5.9 |
| | Advanced MF (stages IIB-IVB) SS | 12 | 6/6 | 61.5 | 33 (26) | 53 | 5.3 |
| | PCCD30+LPDs | 13 | 10/3 | 48.7 | 27 (17) | 11 | 4.3 |
| | Other CTCLs | 14 | 5/9 | 51.1 | 34 (24) | 28 | 3.9 |
| CBCLs | PCFCL | 10 | 6/4 | 59.2 | 25 (22) | 15 | 4.8 |
| | PCMZL | 9 | 3/6 | 64.3 | 14 (10) | 8 | 6 |
| | PCDLBCL, LT | 3 | 2/1 | 77.4 | 3 (0) | 2 | 3.2 |

CBCLs: cutaneous B-cell lymphomas; CTCLs: cutaneous T-cell lymphomas; F: female; M: male; MF: mycosis fungoides; PCCD30+LPDs: primary cutaneous CD30+ T-cell lymphoproliferative disorders; PCDLBCL, LT: primary cutaneous large B-cell lymphoma, leg type; PCFCL: primary cutaneous follicle center lymphoma; PCMZL: primary cutaneous marginal zone lymphoma; SS: Sézary syndrome.

**Table 2. Characteristics of the benign cutaneous lymphoid infiltrates cases**

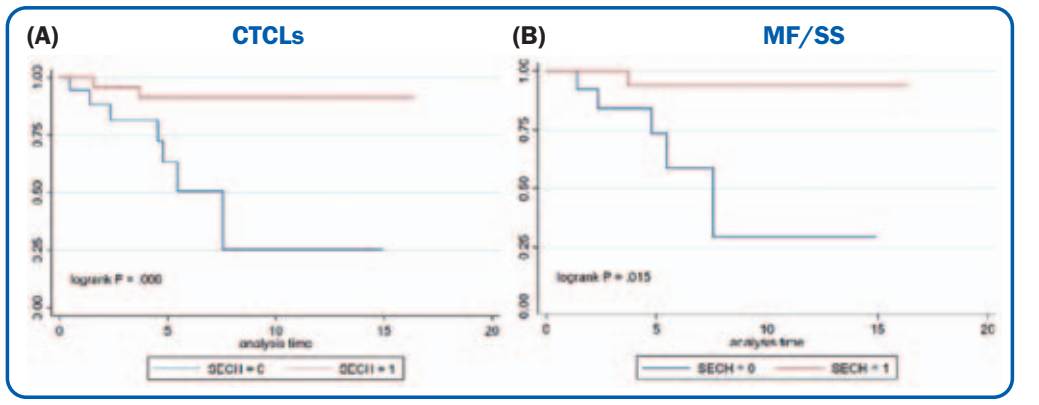
| | N | Gender (M/F) | Age at 1st visit (mean in years) | Skin biopsies (sequential biopsies) | Blood samples | Follow-up (mean in years) |
|---|---|---|---|---|---|---|
| BID | 109 | 67/42 | 58.7 | 130 (27) | 33 | 3.9 |
| Erythroderma | 7 | 4/3 | 72.1 | 25 (20) | 14 | 3.4 |
| Digitate dermatosis / Parapsoriasis | 27 | 25/2 | 61.7 | 43 (21) | 14 | 4.5 |
| CLH | 20 | 10/10 | 55.2 | 26 (12) | 8 | 3.2 |

BID: benign inflammatory dermatoses; CLH: cutaneous lymphoid hyperplasia.

## Results

The BIOMED-2 PCR protocol is a useful method in order to distinguish CTCLs from benign T-cell-rich infiltrates in the skin with high sensitivity (96%) and meaningful specificity (84%) (Table 3). The BIOMED-2 method has also been helpful in differentiating CBCLs from benign cutaneous B-cell infiltrates with very high sensitivity (100%) and reasonable specificity (77%) (Table 4). Among the three multiplex PCR for identifying complete *IGH* gene rearrangements, *IGH* tube A VH FR1-JH disclosed the highest clonal detection rate (66.7%). Clonal heterogeneity between sequential or different skin sites biopsies was identified in 5% of CTCLs and 17% of CBCLs. Detection of the same dominant TCR gene rearrangement in skin and extracutaneous samples is associated with higher disease-specific mortality rates in CTCLs (p= 0.006) and MF/SS patients (p=0.015) (Figure 1).

**Figure 1. Graph showing Kaplan-Meier survival estimates given by the presence or absence of skin - extracutaneous clonal heterogeneity in CTCLs (A) and MF/SS patients (B)**




SECH: skin - extracutaneous clonal heterogeneity. 0: absent; 1: present.

**Table 3. Diagnostic utility of *TCRG* and/or *TCRB* in T-cell cutaneous lymphoproliferations**

| Diagnostic Test - BIOMED-2 PCR Assay | CTCLs *vs* BID + parapsoriasis + erythroderma | MF/SS *vs* BID + parapsoriasis + erythroderma | MF (stages IA-IIA) *vs* BID + parapsoriasis |
|---|---|---|---|
| ***TCRG* gene rearrangements** | Sen: 82.4 (71.2-90.5)<br>Spe: 90.1 (83-94.9)<br>PPV: 83.6 (72.5-91.5)<br>NPV: 89.3 (82-94.3) | Sen: 85.1 (71.7-93.8)<br>Spe: 90.1 (83-94.9)<br>PPV: 78.4 (64.7-88.7)<br>NPV: 93.5 (87-97.3) | Sen: 80 (63.1-91.6)<br>Spe: 90.1 (83-94.9)<br>PPV: 71.8 (55.1-85)<br>NPV: 93.5 (87-97.3) |
| ***TCRB* gene rearrangements** | Sen: 82.4 (71.2-90.5)<br>Spe: 90.1 (83-94.9)<br>PPV: 83.6 (72.5-91.5)<br>NPV: 89.3 (82-94.3) | Sen: 83 (69.2-92.4)<br>Spe: 90.1 (83-94.9)<br>PPV: 78 (64-88.5)<br>NPV: 92.6 (85.9-96.7) | Sen: 80 (63.1-91.6)<br>Spe: 90.1 (83-94.9)<br>PPV: 71.8 (55.1-85)<br>NPV: 93.5 (87-97.3) |
| **Combined *TCRG* +*TCRB* gene rearrangements*** | **Sen: 95.6 (87.6-99.1)**<br>**Spe: 83.8 (75.6-90.1)**<br>PPV: 78.3 (67.9-86.6)<br>**NPV: 96.9 (91.1-99.4)** | **Sen: 97.9 (88.7-99.9)**<br>**Spe: 83.8 (75.6-90.1)**<br>PPV: 71.9 (59.2-82.4)<br>**NPV: 98.9 (94.2-100)** | **Sen: 97.1 (85.1-99.9)**<br>**Spe: 83.8 (75.6-90.1)**<br>PPV: 65.4 (50.9-78)<br>**NPV: 98.9 (94.2-100)** |

NPV: negative predictive value (%); PPV: positive predictive value (%); Sen: sensitivity (%); Spe: specificity (%). (CI95). Data marked in colour are statistically different ($p < 0.05$). *At least clonal detection in one test.

**Table 4. Diagnostic utility of *IGH* and/or *IGK* in distinguishing CBCLs from CLH**

| Diagnostic Test - BIOMED-2 PCR Assay | CBCLs *vs* CLH | PCFCL *vs* CLH | PCMZL *vs* CLH |
|---|---|---|---|
| **Complete *IGH* gene rearrangements: VH–JH** | Sen: 90.9 (70.8-98.9)<br>Spe: 94.1 (71.3-99.9)<br>PPV: 95.2 (76.2-99.9)<br>NPV: 88.9 (65.3-98.6) | Sen: 100 (69.2-100)<br>Spe: 94.1 (71.3-99.9)<br>PPV: 90.9 (58.7-99.8)<br>NPV: 100 (79.4-100) | Sen: 88.9 (51.8-99.7)<br>Spe: 94.1 (71.3-99.9)<br>PPV: 88.9 (51.8-99.7)<br>NPV: 94.1 (71.3-99.9) |
| **Incomplete *IGH* gene rearrangements: DH–JH** | Sen: 26.7 (7.79-55.1)<br>Spe: 90.9 (58.7-99.8)<br>PPV: 80 (28.4-99.5)<br>NPV: 47.6 (25.7-70.2) | Sen: 14.3 (0.4-57.9)<br>Spe: 90.9 (58.7-99.8)<br>PPV: 50 (1.3-98.7)<br>NPV: 62.5 (35.4-84.8) | Sen: 50 (11.8-88.2)<br>Spe: 90.9 (58.7-99.8)<br>PPV: 75 (19.4-99.4)<br>NPV: 76.9 (46.2-95) |
| ***IGK* gene rearrangements: Vk–Jk and Kde** | Sen: 63.6 (30.8-89.1)<br>Spe: 85.7 (42.1-99.6)<br>PPV: 87.5 (47.3-99.7)<br>NPV: 60 (26.2-87.8) | Sen: 100 (47.8-100)<br>Spe: 85.7 (42.1-99.6)<br>PPV: 83.3 (35.9-99.6)<br>NPV: 100 (54.1-100) | Sen: 25 (0.6-80.6)<br>Spe: 85.7 (42.1-99.6)<br>PPV: 50 (1.3-98.7)<br>NPV: 66.7 (29.9-92.5) |
| **Combined complete *IGH* + incomplete *IGH* + *IGK* gene rearrangements*** | Sen: 100 (84.6-100)<br>Spe: 76.5 (50.1-93.2)<br>PPV: 84.6 (65.1-95.6)<br>NPV: 100 (75.3-100) | Sen: 100 (69.2-100)<br>Spe: 76.5 (50.1-93.2)<br>PPV: 71.4 (41.9-91.6)<br>NPV: 100 (75.3-100) | Sen: 100 (66.4-100)<br>Spe: 76.5 (50.1-93.2)<br>PPV: 69.2 (38.6-90.9)<br>NPV: 100 (75.3-100) |

*At least clonal detection in one test.

## Conclusions

The BIOMED-2 PCR protocol has proved to be a useful diagnostic strategy, with some prognostic implications, for studying patients with cutaneous lymphoproliferative processes in the context of clinical, histologic and immunophenotypic data. This technique is particularly valuable in challenging diagnostic scenarios in clinical practice such as: the distinction between early-stages of MF and BID or parapsoriasis and indolent CBCLs and CLH.

## References

1. van Dongen JJ, Langerak AW, Brüggemann M, Evans PA, Hummel M, Lavender FL, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia* 2003;17:2257-317.
2. Sandberg Y, Heule F, Lam K, Lugtenburg PJ, Wolvers-Tettero IL, van Dongen JJ, Langerak AW. Molecular immunoglobulin/T-cell receptor clonality analysis in cutaneous lymphoproliferations. Experience with the BIOMED-2 standardized polymerase chain reaction protocol. *Haematologica* 2003;88:659-70.
3. Lukowsky A, Muche JM, Möbs M, Assaf C, Humme D, Hummel M, Sterry W, Steinhoff M. Evaluation of T-cell clonality in archival skin biopsy samples of cutaneous T-cell lymphomas using the biomed-2 PCR protocol. *Diagn Mol Pathol* 2010;19:70-7.
4. Morales AV, Arber DA, Seo K, Kohler S, Kim YH, Sundram UN. Evaluation of B-cell clonality using the BIOMED-2 PCR method effectively distinguishes cutaneous B-cell lymphoma from benign lymphoid infiltrates. *Am J Dermatopathol* 2008;30:425-30.
5. Willemze R, Cerroni L, Kempf W, Berti E, Facchetti F, Swerdlow SH, Jaffe ES. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. *Blood* 2019;133:1703-1714.

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