
The value of molecular diagnostics in primary cutaneous B-cell lymphomas in the context of clinical findings, histology, and immunohistochemistry

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Background: Primary cutaneous B-cell lymphoma (PCBCL) is classified into 3 major subtypes: primary cutaneous follicle center lymphoma (PCFCL); primary cutaneous marginal zone B-cell lymphoma (PCMZL); and primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT). Diagnosis of PCBCL is mainly based on clinical and (immuno)-histochemical grounds.

Objective: We investigated the diagnostic value of the BIOMED-2 Concerted Action BMH4 CT98-3936 protocol in PCBCL.

Methods: We analyzed with the BIOMED-2 Concerted Action BMH4 CT98-3936 protocol skin specimens from patients with well-defined clinical and (immuno)-histologic PCBCL (n = 18) in comparison with benign lymphocytic infiltrates (n = 9). For molecular staging we also investigated 13 extracutaneous samples from 6 patients with PCLBCL, LT. Each sample was investigated at least twice.

Results: Monoclonality was detected in all of 5 PCFCL; 5 of 6 PCMZL; all of 6 PCLBCL, LT; and 2 of 9 benign lymphocytic infiltrates. In 5 of 6 patients with PCLBCL, LT, a clone corresponding to the clone detected in the skin was detected in 3 of 5 bone-marrow, 4 of 5 blood, and 1 of 3 lymph node specimens. DNA amplification using tubes A and B of IgH was not possible in PCFCL/PCMZL, benign lymphocytic infiltrates, and extracutaneous specimens of PCLBCL, LT, even after repeated analysis up to 11 times. Pseudomonoclonality was identified by repeated analyses in one case of PCMZL and in one case of benign lymphocytic infiltrate.

Limitations: A multicentric, randomized, blinded study is necessary to confirm our results.

Conclusion: Molecular diagnosis supports the clinical and (immuno)-histologic diagnosis in PCBCL. In PCLBCL, LT, molecular staging may be useful. Tubes C through E of IgH and Igκ analyses seem to be superior to tubes A and B of IgH. Each sample should be analyzed at least twice to assess the possibility of pseudomonoclonality. (J Am Acad Dermatol 2011;64:135-43.)

Key words: benign lymphocytic infiltrate; BIOMED-2; IgH rearrangement; Igκ; rearrangement; molecular biology; primary cutaneous B-cell lymphoma.

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The World Health Organization—European Organization for the Research and Treatment of Cancer (EORTC) classification system distinguishes 3 major types of primary cutaneous B-cell lymphoma (PCBCL): primary cutaneous follicle center lymphoma (PCFCL); primary cutaneous marginal zone B-cell lymphoma (PCMZL); and primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT).¹ The diagnosis of PCBCL is based on clinical, histologic, immunohistologic, and molecular criteria. PCBCL is staged according to the recently

published TNM staging system for primary cutaneous lymphoma other than mycosis fungoides and Sézary syndrome.²

PCFCLs present with solitary or grouped plaques or tumors, which are localized on the scalp, forehead, or trunk, less commonly on the legs and normally not on the upper extremities.^{3,4} PCMZLs comprise cases with red to violaceous nodules, papules, or plaques, distributed on the trunk and extremities. Some cases of PCMZL have been associated with *Borrelia burgdorferi* infection.^{5,6} PCLBCL, LTs are common in elderly, female patients (men:women \approx 1:2) and grow rapidly as red or bluish-red tumors mainly on the lower extremities.^{3,7,8} Although PCFCLs and PCMZLs have an excellent prognosis with a 5-year survival of 95% and 100%, respectively, PCLBCL, LTs have a 5-year survival of 55%.³ Histologically PCFCL is a tumor of follicular center cells and shows a mixture of centrocytes (small and large cleaved follicular center cells), centroblasts, and a few reactive T cells.^{3,9} The growth pattern is follicular, diffuse, or follicular and diffuse. PCMZL shows a nodular to diffuse growth pattern of small lymphocytes (centrocyte-like cells), lymphoplasmacytoid and plasma cells admixed with centroblasts and many reactive T cells. PCLBCL, LT tumors are confluent sheets of large centroblasts and immunoblasts with a few small B cells, reactive T cells, or both. Immunohistologically all 3 PCBCLs are positive for the B-cell markers CD20 and CD79a. PCFCL tumor cells stain with surface immunoglobulin and B-cell lymphoma (bcl)-6. In contrast, bcl-2 and multiple myeloma oncogene (MUM)-1 are most often negative. PCMZL are bcl-2⁺, bcl-6⁺, and show immunoglobulin light chain expression of the plasma cells. PCLBCL, LT are bcl-2⁺, MUM-1⁺, and mainly bcl-6⁺. In some cases light chain restriction can be detected by immunohistochemistry and supports the monoclonal nature of PCBCL. More sensitive techniques to detect monoclonality are molecular analyses. This can be done by Southern blot or polymerase chain reaction (PCR) analyses.

Southern blot analyses are expensive, laborious, and time-consuming, and more DNA is needed in

contrast to PCR techniques.¹⁰ However, sensitivity and specificity of the different PCR techniques differ because of ineffective binding of primers especially in somatic hypermutations (SHM), which are common in primary cutaneous lymphoma.¹¹⁻¹⁵ In addition, sensitivity decreases if the distance between the primers is longer than 300 base pair (bp). False-

positive results are common especially in extranodal tissue (eg, skin) where only small amounts of malignant B cells may be present; this is especially true if a sample is analyzed only once. In such circumstances, repeated analyses can help assess the possibility of pseudomonoclonality.¹⁶⁻¹⁸

To improve molecular diagnosis of primary cutaneous lymphoma, the BIOMED-2 Concerted Action BMH4 CT98-3936 protocol (BIOMED-2) was developed by a collaborative study group in 2003.¹⁹ BIOMED-2 is said to have high sensitivity and specificity because it evaluates almost all possible gene rearrangements. It detects VH-JH (tubes A-C) and DH-JH gene rearrangements of IgH (tubes D-E), and Igk

gene rearrangements (tube A: Vk-Jk; tube B: Igk; Vk/intron-Kde) and Igλ gene rearrangements (tube Vλ-Jλ). The value of this protocol was shown in systemic B-cell and T-cell malignancies in large multicenter studies.^{15,20,21} Recently, it was shown that BIOMED-2 can differentiate between benign lymphoid infiltrates in the skin and PCFCL/PCMZL.²² However, sensitivity differs in the subgroups of PCBCL and in the studies.^{22,23} Furthermore, it has not been determined if molecular staging might be possible for PCLBCL, LT, which has a more aggressive course. We sought to investigate the value of molecular techniques for making the diagnosis of PCBCL in the context of clinical findings, histology, and immunohistochemistry. Furthermore, we wanted to identify cases in which BIOMED-2 might aid in staging PCBCL.

METHODS

Patients

We investigated 18 patients with a well-defined PCBCL and 9 patients with benign lymphocytic

CAPSULE SUMMARY

- In our study, the value of the BIOMED-2 Concerted Action BMH4 CT98-3936 protocol for diagnosis/staging of primary cutaneous B-cell lymphoma depended on the subtype.
- In primary cutaneous follicle center/marginal zone B-cell lymphoma, molecular analyses are not needed for routine use but may help differentiate benign lymphocytic infiltrates from primary cutaneous follicle center/marginal zone B-cell lymphoma.
- In primary cutaneous diffuse large B-cell lymphoma, leg type, the BIOMED-2 Concerted Action BMH4 CT98-3936 protocol may be a useful tool for staging extracutaneous compartments.
- Each sample should be analyzed at least twice to assess the possibility of pseudomonoclonality.

Abbreviations used:

bcl-2:	B-cell lymphoma-2
bcl-6:	B-cell lymphoma-6
BIOMED-2:	BIOMED-2 Concerted Action BMH4 CT98-3936
EORTC:	European Organization for the Research and Treatment of Cancer
MUM-1:	multiple myeloma oncogene-1
PCBCL:	primary cutaneous B-cell lymphoma
PCFCL:	primary cutaneous follicle center lymphoma
PCLBCL, LT:	primary cutaneous diffuse large B-cell lymphoma, leg type
PCMZL:	primary cutaneous marginal zone B-cell lymphoma
PCR:	polymerase chain reaction
SHM:	somatic hypermutations
MUM-1:	multiple myeloma oncogene-1

infiltrates who were given a diagnosis between January 2000 and April 2008 in the Departments of Pathology and Dermatology, Venerology, and Allergology, University Medicine Mannheim, Germany (Table I; available online at www.eblue.org). Some patients were excluded because the differentiation between PCMZL and PCFCL was not possible by clinic and (immuno)-histochemistry.

Five of 18 patients with PCBCL were given a diagnosis of PCFCL (28%), 7 of 18 PCMZL (39%) and 6 of 18 PCLBCL, LT (33%). Six of 9 patients with benign lymphocytic infiltrates had cutaneous lymphoid hyperplasia, two had lymphocytic infiltration of the skin Jessner-Kanoff, and one had angiolymphoid hyperplasia with eosinophilia. In patients with PCBCL a nodal or systemic involvement was excluded by bone-marrow biopsy, gastroscopy, colonoscopy, and a total body computed tomography scan. Clinically enlarged lymph nodes were biopsied and analyzed for lymphoma infiltration. Follow-up was done every 3 months. The patients were staged according to the recently published TNM classification system² and treated according to the German²⁴ and EORTC²⁵ treatment guidelines for cutaneous lymphomas.

Histology/Immunohistochemistry. The following antibodies were used: ki-67 (clone MIB-1, Dako, Eching, Germany, 1:800), CD20 (clone L26, Dako, 1:2000), CD79a (clone JCB117, Dako, 1:200), bcl-2 (clone 124, Dako, 1:500), bcl-6 (clone PG-B6, Dako, 1:10), and MUM-1 (clone Mum1p, Dako, 1:200). The staining was valued negative if it was described as “less than 50% of the tumor cells,” “weak staining,” or “isolated.” The results were positive if the result was described as “more than 50% of the tumor cells” or “diffuse.” Photodocumentation of the (immuno)-histochemistry was taken with an Olympus camera C-4040 using an Olympus BX51 microscope

with magnifications between $\times 10$ and $\times 40$ (Olympus, Hamburg, Germany).

Molecular biology. Clonality was detected in formalin-fixed (paraffin-embedded) specimens according to PCR-based BIOMED-2.¹⁹ In general 100 μ g of DNA were used. For the primer sets A and B of BIOMED-2 for IgH up to 500 μ g of DNA were used in all cases where amplification could not be achieved with 100 μ g. To avoid pseudomonoclonality each sample was tested at least twice. Individual samples were analyzed up to 11 times to get a DNA amplification. Monoclonal PCBCL tumor samples were used as positive controls for all cases of benign lymphocytic infiltrates. To economize and to prove clonality as fast as possible we stopped searching for clonality if clonality was detected by repeated analyses with at least one of the primer sets A through E.^{11,18} In cases with no detection of monoclonality we performed analyses with BIOMED-2 for Ig κ . PCLBCL, LT tumors show a more aggressive clinical course than PCBCL with indolent behavior. In contrast to PCBCL with indolent behavior, local therapy is most often not sufficient to control the disease and even inconspicuous staging does not exclude recurrent, systemic involvement with lethal outcome. Therefore, extracutaneous specimens (lymph node, blood, and bone marrow) of PCLBCL, LT were analyzed when available.

RESULTS

Study population

Of our 18 patients with PCBCL 5 were given a diagnosis of PCFCL (28%), 7 of PCMZL (39%), and 6 of PCLBCL, LT (33%). Although patients with PCFCL and PCMZL were aged 56 (37-66) and 54 (44-66) years, respectively, the average patient given a diagnosis of PCLBCL, LT was aged 83 (74-87) years (Table I; available online at www.eblue.org). In PCFCL the primary cutaneous locations were trunk and head/neck area. There were no lesions on the extremities. In contrast, skin lesions of our patients with PCMZL did not demonstrate any predilection and all PCLBCL, LT tumor lesions were located on the lower legs. Although most PCFCL and PCMZL lesions were smaller than 5 cm in size, PCLBCL, LT tumors showed a great variation in size (<5 to >30 cm).

All patients were staged according to the recently published TNM staging classification.² Eight patients were staged T1, 9 patients were staged T2, and one patient with PCMZL was staged T3a at the time of diagnosis (Figs 1 and 2, and Table I; available online at www.eblue.org). Even if the diagnosis PCBCL provides that bone-marrow and lymph node involvement is excluded and therefore N and M should

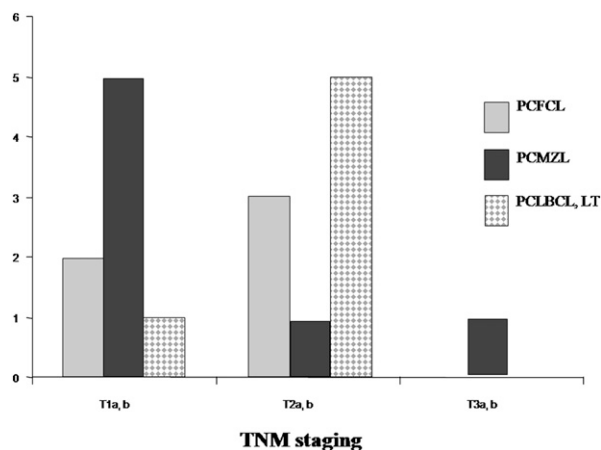


Fig 1. TNM stages of different primary cutaneous B-cell lymphomas (PCBCLs) at time of diagnosis. Patients with PCBCL with indolent behavior were more often staged T1a,b than patients with primary cutaneous diffuse large B-cell lymphoma, leg type (*PCLBCL, LT*), who were more commonly staged T2a,b. *PCFCL*, Primary cutaneous follicle center lymphoma; *PCMZL*, primary cutaneous marginal zone B-cell lymphoma.

be 0 at the time of diagnosis, we included two patients with N1 at the time of diagnosis. These patients were given a diagnosis of PCLBCL, LT and had far progressed skin lesions and histologically an early infiltration of the inguinal lymph nodes. Three cases of PCFCL (1, 3, and 5), two PCMZL cases (9 and 10), and one case of PCLBCL, LT (14) showed progressive skin disease during follow-up. As recommended all of our patients with PCBCL with indolent behavior (PCFCL and PCMZL) were treated with surgical excision combined with local radiotherapy.^{24,26} Most patients with PCLBCL, LT received rituximab (anti-CD20 antibody) in combination with surgical excision and local radiotherapy.

The median time of follow-up was 33.33 ± 34.54 months. Four patients with PCFCL and 3 patients with PCMZL had recurrent disease, including one patient with 3 recurrences (patient 9). None of the patients with PCFCL or PCMZL died during the study. In PCLBCL, LT, two of 6 patients died as a result of the lymphoma, two because of a different cause, and two are still alive. The median time of survival for patients with PCLBCL, LT was 14.33 ± 28.38 months.

Immunohistochemistry. The B-cell origin of the tumor could be shown in all cases with one of the B-cell markers CD20 or CD79. PCFCL tumor cells showed overexpression of the antiapoptotic protein bcl-6 (Fig 3, A). One patient showed overexpression of bcl-2 (patient 1). None of the PCFCL lesions expressed MUM-1. Light chain restriction was analyzed in 4 cases; one patient showed Ig λ light chain restriction.

In PCMZL cases tumor cells expressed bcl-2 (Fig 3, B). Bcl-6 was expressed once and MUM-1 in no case. One patient showed Ig κ light chain restriction. PCLBCL, LT tumor cells stained positive for bcl-2 in all cases at the beginning of the disease (Fig 3, C). Two of 6 patients with PCLBCL, LT showed overexpression of bcl-6 and 4 of 6 patients stained positive for MUM-1 (Fig 3, D). One case showed Ig κ light chain restriction.

Molecular biology. In 17 of 18 patients with PCBCL and all of 9 patients with benign lymphocytic infiltrates molecular analyses were performed with BIOMED-2 for IgH. In one case of PCMZL no material of the tumor was available (patient 6). Monoclonality could be detected with BIOMED-2 for IgH in 10 of 17 PCBCL tumor samples and in none of 9 samples of benign lymphocytic infiltrates (Tables II and III; available online at www.eblue.org). Monoclonality was diagnosed after at least two repeated identifications of the same clone in the same DNA sample or a second DNA sample from the same specimen. However, sensitivity of BIOMED-2 for IgH varied in PCBCL subgroups.

In PCLBCL, LT, monoclonality was seen in 5 of 6 cases with BIOMED-2 for IgH. In PCFCL and PCMZL, monoclonality could be detected 3 of 5 and 2 of 6 times with BIOMED-2 for IgH. In the remaining 7 of 17 cases and all of 9 cases of benign lymphocytic infiltrates we could not analyze IgH rearrangement with the primers A and B because DNA amplification was not possible even after repeated analyses of up to 11 times in contrast to monoclonal control samples run in the same PCR. Tube C detected monoclonality 5 of 11 times in PCBCL with indolent behavior. In contrast tubes D and E showed several times either polyclonality (Fig 4, A) or pseudomonoclonality (Fig 4, B) in PCBCL with indolent behavior. Pseudomonoclonal cases either showed two monoclonal results with different product sizes by repeated analyses of the same DNA or one monoclonal and one polyclonal result of the same DNA sample. Therefore, Ig κ analyses with BIOMED-2 were performed in 7 of 11 PCBCL with indolent behavior; 3 PCLBCL, LT; and all of 9 benign lymphocytic infiltrates. In PCBCL with indolent behavior, monoclonality could be detected with BIOMED-2 for Ig κ (tubes A and B) in all of 7 cases (Fig 5 and Table II; available online at www.eblue.org). In PCLBCL, LT, monoclonality could be detected all of 3 times with BIOMED-2 for Ig κ . In benign lymphocytic infiltrates BIOMED-2 for Ig κ (tube A) detected 6 of 9 times pseudomonoclonality, and 2 of 9 times monoclonality.

In all of 6 cases of PCLBCL, LT, 13 samples of extracutaneous tissue were analyzed (3 lymph node, 5 bone-marrow, and 5 peripheral blood specimens).

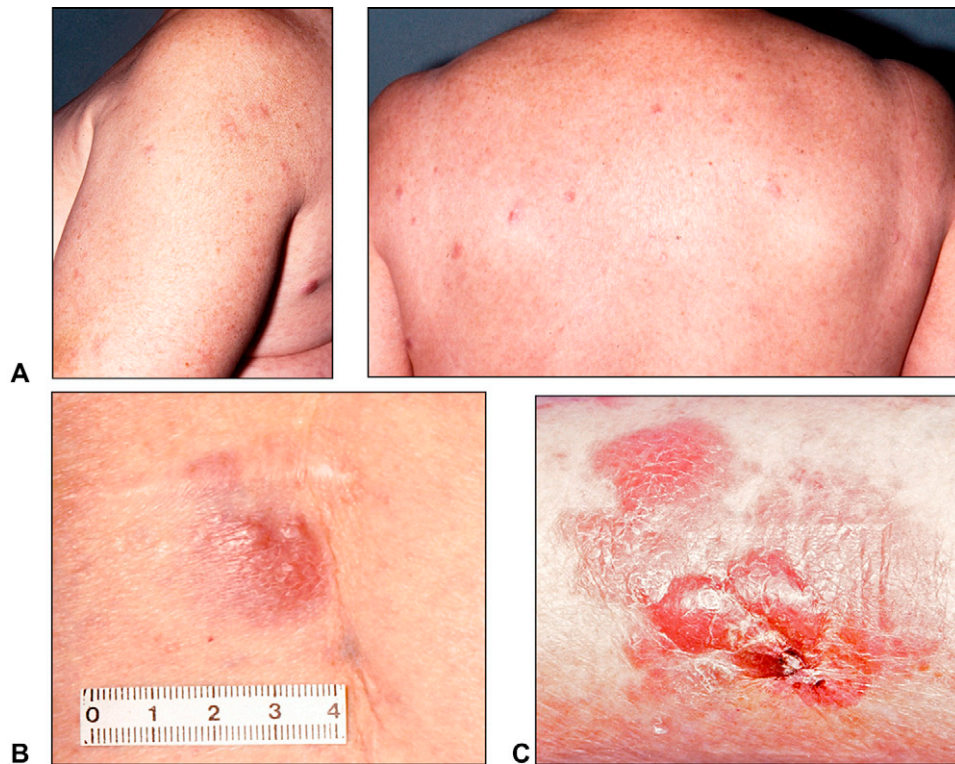


Fig 2. Different clinical pictures of TNM stages. **A**, Multiple erythematous nodules on upper aspect of back and left shoulder (patient 10, primary cutaneous marginal zone B-cell lymphoma). She was staged T2cN0M0 (regional skin involvement: multiple lesions limited to one body region or two contiguous body regions: all disease encompassing >30-cm-diameter circular area). **B**, Erythematous nodule on right lower aspect of back recurred after patient received surgical excision without radiotherapy (patient 1, primary cutaneous follicle center lymphoma). She was staged T2bN0M0 because tumor had primarily been found on upper aspect of back as well (regional skin involvement: multiple lesions limited to one body region or two contiguous body regions: all disease-encompassing >15 and <30-cm-diameter circular area). **C**, Erythematous nodule on erythematous, well-demarcated plaque on right lower leg (patient 17). Patient was also staged T2bN0M0 because she had more than one lesion (regional skin involvement: multiple lesions limited to one body region or two contiguous body regions: all disease encompassing >15 and <30-cm-diameter circular area).

In extracutaneous tissue DNA amplification was in 8 cases not possible with BIOMED-2 for IgH tubes A or B. In contrast, DNA amplification was always possible with tubes C-E of IgH and tubes A-B of Igκ. In 5 of 6 patients identical clones could be detected in the skin and extracutaneous tissue by repeated analyses (3/5 times in the bone marrow, 4/5 times in peripheral venous blood, and 1/3 times in lymph node tissue) (Fig 4, C and D). Although in 4 patients the clone could be detected in just one of the extracutaneous compartments, patient 17 showed the skin clone in blood and bone marrow. In 5 analyses the clone detected in the skin and in extracutaneous tissue varied and was therefore judged as a clone of undetermined significance (2 × bone-marrow, 2 × lymph node, 1 × blood specimens).¹⁶⁻¹⁸

Interestingly, the patient, who did not show monoclonality in extracutaneous specimens had

one small nodule and was staged T1aN0M0. The other 5 patients had clinically progressive disease with more than one lesion and were staged T2aN0M0 or higher.

DISCUSSION

Our BIOMED-2 PCR-based assays were performed in 17 of 18 well-characterized PCBCL tumors and in all of 9 benign lymphocytic infiltrates. Monoclonality was demonstrated in 16 of 17 analyzed PCBCL cases (5/5 PCFCL; 5/6 PCMZL; and 6/6 PCLBCL, LT) and in 2 of 9 cases with benign lymphocytic infiltrates. In one case of PCMZL monoclonality could not be detected with BIOMED-2. However, light chain restriction was found in this case by immunohistochemistry. Furthermore, primer panels A through D of BIOMED-2 for IgH could not amplify any DNA. Unfortunately, we could not

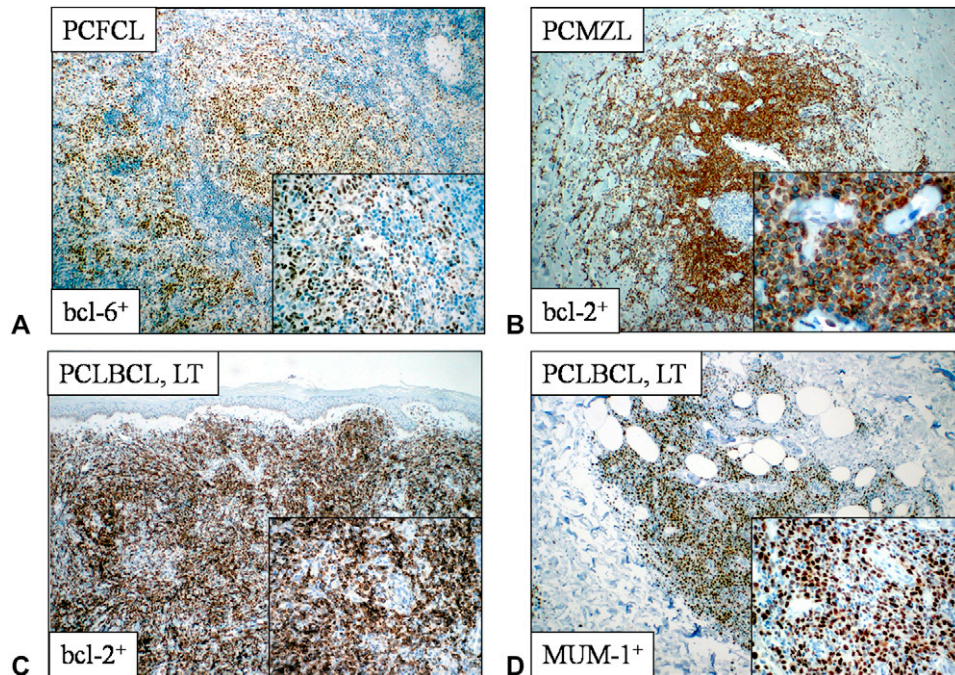


Fig 3. Immunophenotypic features of primary cutaneous B-cell lymphoma. **A**, Intrafollicular primary cutaneous follicle center lymphoma (*PCFCL*) tumor cells stain $bcl-6^{+}$, perifollicular cells are $bcl-6^{-}$ (patient 3, *PCFCL* with follicular growth pattern). **B**, Primary cutaneous marginal zone B-cell lymphoma (*PCMZL*) cells in marginal zone strongly express $bcl-2^{+}$; in germinal center remnants are $bcl-2^{-}$ (patient 10, *PCMZL*). **C**, Primary cutaneous diffuse large B-cell lymphoma, leg type (*PCLBCL, LT*) cells show strong expression of $bcl-2^{+}$, in grenz zone no cells express $bcl-2$ (patient 17, *PCLBCL, LT*). **D**, *PCLBCL, LT*. Most of tumor cells are $MUM-1^{+}$ (patient 15, *PCLBCL, LT*).

analyze this tumor with BIOMED-2 for $Ig\kappa$ because of lack of material. We used paraffin-embedded specimens and it is widely accepted that this does not influence sensitivity or specificity.^{12,27} However, all samples were archival skin specimens, which are more difficult to analyze than freshly prepared samples.^{12,23,28} The reason for this could be DNA degradation, which is more common in older specimens especially if they are formalin fixed, which causes irreversible DNA changes.

The decision to perform molecular techniques must be made in the context of the clinical findings and (immuno)-histochemistry. If PCBCl with indolent behavior is suspected and benign lymphocytic infiltrates cannot be excluded by clinical evaluation, histology, and immunohistochemistry, BIOMED-2 can be a valuable differentiating tool.²² Morales et al²² detected monoclonality in 22 of 26 cases of PCBCl with indolent behavior. We consistently detected monoclonality in 10 of 11 cases of PCBCl with indolent behavior. In contrast, in benign lymphoid infiltrates a monoclonal clone was just detected in 1 of 23 cases²² and in 2 of 9 cases in our study. However, both studies show that molecular

analyses have to be interpreted in the context of the clinical findings and (immuno)-histochemistry.

In our analyses primers A and B were not able to amplify any DNA in 6 skin specimens of PCBCl with indolent behavior or in 13 skin specimens of 9 patients with benign lymphocytic infiltrates despite repeated analyses with different DNA concentrations. This is in line with the report of Lukowski et al,²³ who could not amplify any DNA with tube A in 77% (21/27) and tube B in 66% (18/27) of their PCBCl specimens. For PCBCl the reason could be SHM. In B-cell receptor development in the germinal center, SHM occur especially in complementary-determining regions that are segments of the V_H region. It is speculated that for tumor cells with an elevated rate of SHM (eg, follicular tumors), BIOMED-2 for $IgH V_H$ through J_H (tubes A-C) might not be sufficient to determine clonality.¹⁹ However, tube C worked well in our hands with only one failure of DNA amplification. Therefore, we believe that it is not the whole $IgH V_H$ through J_H region where molecular changes make it difficult to amplify DNA with BIOMED-2. Furthermore, tube C detects amplicons between 70 and 130 in contrast to tube

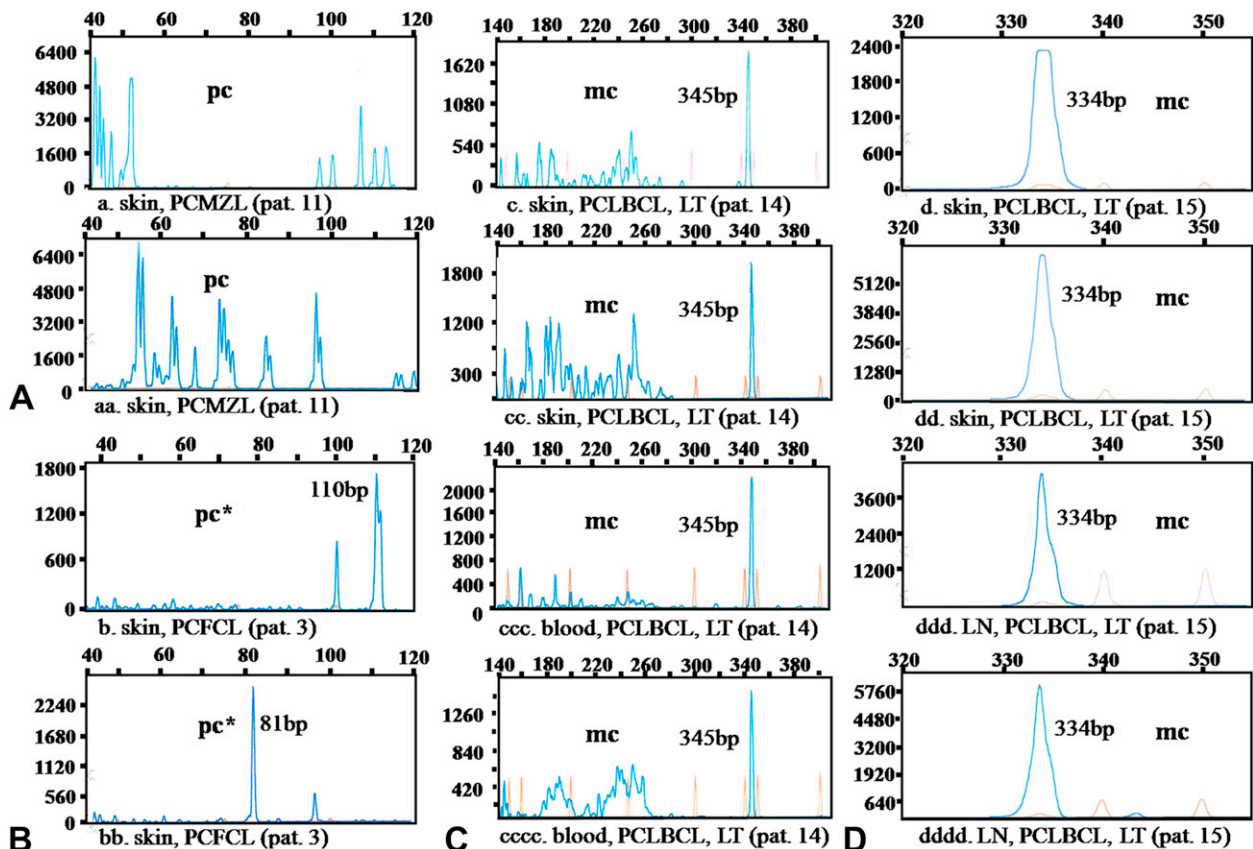


Fig 4. Examples of molecular analyses with BIOMED-2 Concerted Action BMH4 CT98-3936 protocol for IgH. **A**, Repeated analyses of same DNA show polyclonal (*pc*) pattern in skin specimen of patient 11 (primary cutaneous marginal zone B-cell lymphoma [PCMZL]). **B**, Repeated analyses of same DNA show two different monoclonal (*mc*)-like spikes (110 and 81 base pair [bp]) in skin specimen of patient 3 (primary cutaneous follicle center lymphoma [PCFCL]). Analyses are interpreted as pseudo-mc (*pmc*). **C**, Repeated analyses of same DNA show *mc* spikes at 345 bp (*c* and *cc*) on two occasions. Analyses could be confirmed with blood specimens (*ccc* and *cccc*). Analyses are interpreted as *mc* B-cell infiltrate in skin and blood (patient 14, primary cutaneous diffuse large B-cell lymphoma, leg type [PCLBCL, LT]). **D**, Repeated analyses of same DNA show *mc* spike at 334 bp (*d* and *dd*). Analyses could be confirmed with lymph node (LN) specimens (*ddd* and *dddd*). Analyses are interpreted as *mc* B-cell infiltrate in skin and LN (patient 15, PCLBCL, LT). *Pseudomonoclonality.

A (310-360 bp) and tube B (250-295 bp), and it is speculated that this might explain the high failure of DNA amplification with tubes A and B.²² In benign lymphocytic infiltrates the B-cell infiltrate is less dense than in PCBCL and this may explain why DNA amplification was not possible in many cases of benign lymphocytic infiltrates. However, Morales et al²² did not have difficulty amplifying DNA with these specific primers. They used formalin-fixed tissue, whereas Lukowski et al²³ and we used paraffin-embedded specimens. This could explain the discrepancy among the 3 studies.

Our Ig κ studies detected clonality in all of 7 PCBCL with indolent behavior. This is in line with earlier studies that showed that the combination of IgH and Ig κ analyses could increase the clonality

detection rate from 69% to 85% in PCBCL.²² Especially in systemic follicular lymphomas and PCFCL, Ig κ analyses are superior to BIOMED-2 for IgH.^{22,28} The reason for this might be the lower rate of SHM in the Ig κ locus and that the average size between the PCR products is smaller in Ig κ BIOMED-2.^{12,20,28} However, both monoclonal results of our benign lymphoid infiltrates were achieved with Ig κ analyses (tube A). Obviously, these primer tubes were more sensitive but less specific in our study.

We recommend BIOMED-2 for patients where PCBCL with indolent behavior and benign lymphocytic infiltrates cannot be differentiated by clinical findings and (immuno)-histochemistry. If BIOMED-2 is necessary, analyses with tubes C through E of IgH

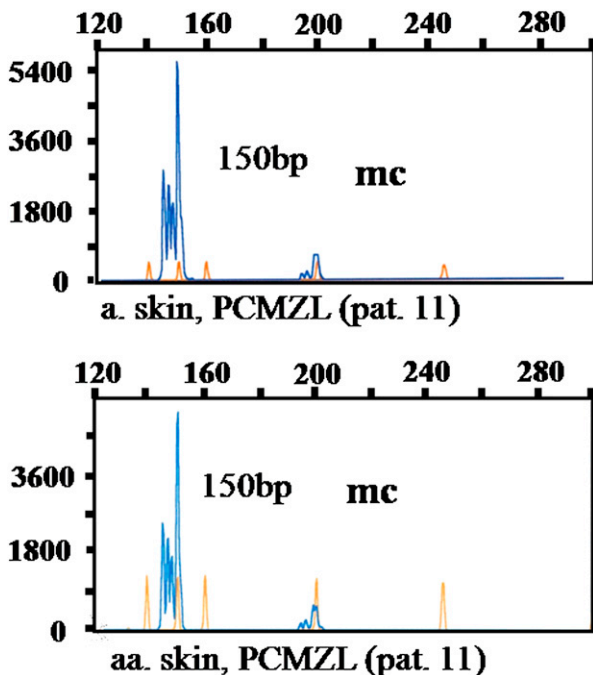


Fig 5. Examples of molecular analyses with BIOMED-2 Concerted Action BMH4 CT98-3936 protocol for Ig κ . Repeated analyses of same DNA show monoclonal (mc) spike at 150 base pair in patient with primary cutaneous B-cell lymphoma with indolent behavior (patient 11, primary cutaneous marginal zone B-cell lymphoma [PCMZL]).

and Ig κ (tubes A and B) seem to be superior to tubes A and B of IgH. BIOMED-2 is time-consuming and expensive, as repeated analyses are necessary to exclude pseudomonoclonality. This is especially true for cases with a sparse lymphocytic infiltrate and is reflected by the high detection rate of pseudomonoclonality in our samples with benign lymphocytic infiltrates. In patients with PCBCL it was always possible to detect monoclonality with Ig κ analyses. However, Ig κ analyses with tube A detected monoclonality in two cases of benign lymphocytic infiltrates. Thus, BIOMED-2 does not seem to be necessary for routine use in PCBCL with indolent behavior, but may help distinguish PCBCL with indolent behavior from benign lymphocytic infiltrates.

In patients with suspected PCLBCL, LT, BIOMED-2 seems to be a valuable tool, and might aid in staging and detecting minimal residual disease after therapy. PCLBCL, LT skin tumors showed monoclonal B-cell infiltrates in all 7 samples of 6 patients. In 5 of 7 skin samples monoclonality was detected with BIOMED-2 for IgH (V_H-J_H) and 4 times monoclonality was detected with BIOMED-2 for Ig κ . Our data are in line with those of Lukowski et al,²³ who detected clonality in 100% of their patients with PCLBCL, LT with BIOMED-2 for IgH (V_H-J_H)

rearrangement. Thirteen extracutaneous samples of 6 patients with PCLBCL, LT were analyzed with BIOMED-2. Similar to PCBCL with indolent behavior and benign lymphocytic infiltrates, tubes A and B of IgH failed several times because DNA amplification was not possible (8/13). With tubes C through E of IgH and tubes A and B of Ig κ it was always possible to amplify DNA. Monoclonality was detected in all extracutaneous samples (100%). In 8 of 13 samples (5 of 6 patients) these clones could be correlated to the clone identified in the skin. One patient even showed the skin clone in two different extracutaneous compartments (blood and bone marrow). In the remaining 5 samples these extracutaneous clones were of undetermined significance.^{11,16,17}

In two cases monoclonality was detected in extracutaneous specimens and pseudomonoclonality in the skin at the time of the diagnosis (blood [patient 15] and bone marrow [patient 16]). In both patients pseudomonoclonality was based on one polyclonal and one monoclonal result. The monoclonal result was identical to the clone detected in the extracutaneous specimens. In patient 15 the remaining analyses showed the same clonality pattern in skin and blood. In patient 16, analyses of the skin tumor at later stages revealed the same product size as detected before in the bone marrow. Therefore, we suggest that repeated biopsies may be necessary if PCBCL is clinically and/or (immuno)-histochemically suspected.

In summary, BIOMED-2 supports the diagnosis of PCLBCL, LT and can detect minimal residual disease in compartments other than the skin. Recently, molecular staging was included in the TNMB staging system for cutaneous T-cell lymphomas.²⁹ Perhaps this will be necessary for PCLBCL, LT as well. Furthermore, our data suggest that PCLBCL, LT often show evidence of minimal residual disease in extracutaneous compartments, which require systemic treatment (eg, with rituximab, cyclophosphamide, Adriamycin, vincristine, and prednisone or doxorubicin). Thus, BIOMED-2 might be helpful for monitoring of minimal residual disease during therapy. However, PCR analyses should always be repeated to exclude false-positive results, ie, pseudomonoclonality.

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Table I. Clinical data of patients with primary cutaneous B-cell lymphoma

No.	Diagnosis	Sex/age, y	Duration of disease, mo	Lesion	No. of skin lesions	Location	Size, Øcm	LN/BM involvement	TNM ²	Therapy	Follow-up, mo	No. of recurrences, outcome
1	PCFCL	F/59	24	Nodule	2	LBB, UB	<5	Ø	T2bN0M0	SE	91	
			2	Plaque	1	LBB	<5	Ø	T2bN0M0	SE + RT		
			6	Patch	2	LBB + C	<5	Ø	T3aN0M0	SE + RT		PD, CR 3
2		M/54	6	Nodule + patch	>10	HN	<5	Ø	T2aN0M0	SE	2	
			8	Nodule	1	HN	5-15	Ø	T2aN0M0	SE + RT		1, CR
3		M/66	ND	Nodule	1	HN	<5	Ø	T1aN0M0	SE + RT	9	
			1	Nodule	1	HN	<5	BM	T1aN0M1	SE + RT + R-CHOP		1, PD
4		M/62	12	Plaque	ND	UB	>15	Ø	T2bN0M0	SE + RT	18	0, CR
5		M/37	8	Nodule	1	HN	<5	Ø	T1aN0M0	SE + RT		
			ND	Nodule	2	C	<5	Ø	T2bN0M0	SE + RT		PD
6	PCMZL	F/62	1	Papule	1	UB	<5	Ø	T1aN0M0	SE + RT	79	0, CR
7		F/44	72	Papule	2	LUA	<5	Ø	T1aN0M0	SE + RT	64	0, CR
8		F/66	5	Papule	1	HN	<5	Ø	T1aN0M0	SE + RT	41	0, CR
9		M/47	ND	Papule	1	AG	ND	ND	T1xNXMX	SE	79	
			9	Papule	5	3UB + 1C + 1RUL	ND	Ø	T3cN0M0	SE + RT		PD
			1	Nodule	2	1RLLF + LUA	<5	ND	T3cNXMX	SE + RT		
			ND	Nodule	3	2UB + 1C	<5	Ø	T3cN0M0	SE + RT		3, CR
10		F/54	18	Papule + nodule	>10	UB, LUA	<5	Ø	T3aN0M0	SE + RT	18	
			1	Papule	1	C	<5	Ø	T3cN0M0	SE + RT		1, PD
11		M/46	18	Nodule	1	RUA	<5	ND	T1aNxMx	SE	85	
			6	Nodule	1	RUA	<5	Ø	T1aN0M0	SE + RT		1, CR
12		M/59	2	Plaque	1	HN	<5	Ø	T2bN0M0	SE + RT	6	0, CR
13	PCLBCL, LT	M/85	2	Nodule	1	LUL	<5	Ø	T1aN0M0	SE + RT	3	
									T1aN0M0	CHOP		
									T1aN0M0	RT		3, D
									T1aN0M0	CHOP		
14		F/74	8	1 Ulcus, 2 nodules	3	RLLF	>15,	Ø	T2bN0M0	R	72	
				Nodule, plaque			<5, 5-15			RT		
			ND		>5, >3	RUL, RLLF	>30	Ø	T2cN0M0	R + RT		PD

Continued

Table I. Cont'd

No.	Diagnosis	Sex/age, y	Duration of disease, mo	Lesion	No. of skin lesions	Location	Size, Øcm	LN/BM involvement	TNM ²	Therapy	Follow- up, mo	No. of recurrences, outcome
15		F/87	4	Plaque	10	LLL + LUL	>15	ND	T2cN0M1			2, DOD
16		F/82	6	Nodule + plaque Nodule	2	LLL	<5	Inguinal LN	T2bN0M0 T2bN1M0	R + SE: LN	8	D
					2	LLL	5-15	Ø	T2bN1M0	SE + RT		1, DOD
17		F/83	12	Nodule	3	RLL	<5, <5, >15, <5, <5	Ø	T2bN0M0	R + RT	1	Still in treatment
18		M/84	2	Nodule	3	RLL	<5, <5, <5	Inguinal LN	T2aN1M0	SE + R + RT	1	Still in treatment

AG, Abdominal and genital; BM, bone marrow; C, chest; CHOP, cyclophosphamide, Adriamycin, vincristine, and prednisone; CR, constant remission; D, died; DOD, died of disease; F, female; HN, head and neck; LBB, lower aspect of back and buttock; LLL, left lower leg and foot; LN, lymph node; LUA, left upper arm; LUL, left upper leg; M, male; ND, not determined; PCFCL, primary cutaneous follicle center lymphoma; PCLBCL, LT, primary cutaneous diffuse large B-cell lymphoma, leg type; PCMZL, primary cutaneous marginal zone B-cell lymphoma; PD, progressive disease; R, rituximab; RLL, right lower leg and foot; RT, local radiotherapy; RUA, right upper arm; RUL, right upper leg; SE, surgical excision; UB, upper aspect of back; Ø, no amplification possible.

Table II. Primary cutaneous B-cell lymphoma: BIOMED-2 Concerted Action BMH4 CT98-3936 protocol gene rearrangement results

No.	Diagnosis		IgH (V _H J _H)			IgH (D _H J _H)		Igκ		Σ
			Tube A (320-355 bp)	Tube B (250-290 bp)	Tube C (120-150 bp)	Tube D (140-410 bp)	Tube E (~120 bp)	Tube A (120-300 bp)	Tube B (210-400 bp)	
1	PCFCL	Skin	ND	ND	mc	ND	pc	ND	mc	mc
2		Skin	Ø	Ø	pc	pc	pc	mc	mc	mc
3		Skin	Ø	Ø	pc	pc	pmc	pc	mc	mc
4		Skin	ND	ND	mc	ND	pc	ND	ND	mc
5		Skin	ND	pmc	mc	mc	mc	ND	ND	mc
7	PCMZL	Skin	Ø	Ø	Ø	Ø	pmc	ND	ND	pmc
8		Skin	ND	ND	mc	mc	pc	pc	mc	mc
9		Skin	ND	pc	mc	ND	mc	ND	ND	mc
10		Skin	Ø	Ø	pc	pc	pc	mc	pc	mc
11		Skin	Ø	Ø	pc	pc	pc	mc	pmc	mc
12		Skin	Ø	Ø	pc	pc	pc	mc	pc	mc
13	PCLBCL, LT	Skin	Ø	mc	pc	mc	pc	pc [†]	mc	mc
		BM	Ø	Ø	pc	ND	pc	ND	ND	pc
14		Skin	mc	pc	mc	mc	pc	ND	ND	mc
		Blood	mc	pc	mc*	pc	pc [†]	ND	ND	mc
		LN	Ø	Ø	pc	mc*	pc	ND	ND	mc*
15		Skin	mc	mc	pc [†]	mc	pc [†]	mc	ND	mc
		LN	mc	mc	pc [†]	mc	mc	mc	ND	mc
16		Skin	ND	pc	pc	mc	pc [†]	ND	ND	mc
		Blood	pc	mc*	mc*	mc	pc	mc*	ND	mc
		BM	Ø	mc*	mc*	pc [†]	pc	ND	ND	mc*
17		Skin	Ø	Ø	pc [†]	pc	pc	mc	ND	mc
		Skin	mc	mc	mc	pc	pc	mc	mc	mc
		Blood	mc	mc	mc	pc	pc	ND	mc	mc
		Blood	pc	Ø	pc	mc*	pc	mc	ND	mc
		BM	Ø	Ø	mc	pc	pc	ND	ND	mc
		BM	Ø	Ø	mc*	mc*	mc*	mc	ND	mc
18		Skin	ND	ND	mc	mc	mc	ND	ND	mc
		Blood	mc*	mc*	pc [†]	mc*	mc*	mc*	ND	mc*
		LN	Ø	Ø	pc	mc*	pc	mc*	ND	mc*
		BM	Ø	Ø	pc	mc	pc [†]	ND	ND	mc

BM, Bone marrow; LN, lymph node; LT, leg type; ND, not determined; mc, monoclonality; pc, polyclonality; PCFCL, primary cutaneous follicle center lymphoma; PCMZL, primary cutaneous marginal zone B-cell lymphoma; pmc, pseudomonoclonality; Ø, no amplification possible.

*Undetermined significance.²¹

[†]Pseudomonoclonality.

Table III. Benign lymphocytic infiltrates: BIOMED-2 Concerted Action BMH4 CT98-3936 protocol gene rearrangement results

No.	Diagnosis		IgH (V _H J _H)			IgH (D _H J _H)		Igκ		Σ
			Tube A (320-355 bp)	Tube B (250-290 bp)	Tube C (120-150 bp)	Tube D (140-410 bp)	Tube E (~120 bp)	Tube A (120-300 bp)	Tube B (210-400 bp)	
1	LI	Skin	Ø	Ø	pc	Ø	pc	pmc	Ø	pc
2		Skin	Ø	Ø	pc	Ø	pc	mc	Ø	mc
3	CLH	Skin	Ø	Ø	pmc	Ø	pc	Ø	Ø	pc
		Skin	Ø	Ø	pc	pmc	pc	Ø	Ø	pc
4		Skin	Ø	Ø	pc	Ø	pc	pmc	Ø	pc
5		Skin	Ø	Ø	pc	Ø	pc	mc	Ø	mc
		Skin	Ø	Ø	pc	Ø	pc	pmc	Ø	pc
6		Skin	Ø	Ø	pc	Ø	pmc	pmc	Ø	pc
7		Skin	Ø	Ø	pmc	Ø	pc	Ø	Ø	pc
8		Skin	Ø	Ø	pmc	Ø	pmc	pmc	Ø	pmc
9	AHE	Skin	Ø	Ø	pmc	Ø	pc	Ø	Ø	pc
		Skin	Ø	Ø	pc	Ø	pc	pmc	Ø	pc
		Skin	Ø	Ø	pmc	Ø	pc	Ø	Ø	pc

AHE, Angiolymphoid hyperplasia with eosinophilia; CLH, cutaneous lymphoid hyperplasia; LI, lymphocytic infiltration of the skin Jessner-Kanoff; mc, monoclonality; pc, polyclonality; Ø, no amplification possible.