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T-cell receptor-γ gene rearrangement analysis in the diagnosis of patients with erythroderma

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Abstract

The diagnosis of erythroderma is challenging, since clinical, histopathological and immunophenotypic findings are insufficient to differentiate between inflammatory and lymphomatous erythroderma. Thus, multiplex PCR was used for T-cell receptor-γ gene rearrangement analysis, in the skin and peripheral blood samples of 24 patients (20 men and 4 women) with erythroderma of varying origin, in order to estimate its diagnostic value. Cutaneous T-cell lymphoma was confirmed in 9, benign inflammatory dermatosis in 12, and idiopathic erythroderma and clonal dermatitis in 3 patients. In the group of patients with erythrodermic cutaneous T-cell lymphoma, the dominant clone was detected in the skin of 8/9, and in none of the patients with inflammatory dermatoses. A dominant clone was found in peripheral blood of 5/6 samples of patients with erythrodermic cutaneous T-cell lymphoma, and in 2/12 patients with inflammatory dermatosis. T-cell receptor-γ gene rearrangement analysis is valuable in differentiation between inflammatory and lymphomatous erythroderma, thus substantially improving the diagnosis of patients with erythroderma.

Erythroderma, or exfoliative dermatitis, is a rare, but severe skin manifestation, involved in several skin disorders. The patient is presenting with erythema of at least 90% of the body skin surface, and varying degree of scaling. In addition, hyperkeratosis of the palms and soles, alopecia and severe pruritus may develop, and vital functions are compromized, due to increased cardiac output, hypoalbuninemia, fever and susceptibility to sepsis, since the skin barrier function is seriously compromised.

At first, the treatment of patient is symptomatic, (correction of electrolyte disbalance and hypoalbuminemia, prophylaxis of the secondary infections, antiinflammatory treatment), but precise diagnosis of the cause of erythroderma is mandatory for proper final treatment.

The etiological diagnosis of erythroderma can often be difficult, bearing in mind that clinical presentation is often non-specific. Psoriatic erythroderma, erythrodermic pityriasis rubra pilaris and erythrodermic cutaneous T-cell lymphoma (CTCL) have their specific features, but sometimes are indistinguishable. Moreover, it is particularly difficult to differentiate between erythrodermic dermatitis (atopic, contact etc.) and cutaneous T-cell lymphoma, which can clinically look alike. In addition, although patch and plaque stage mycosis fungoides have clear histologic criteria, their erythrodermic stages of histopathological features are often non-diagnostic, so the diagnosis of erythrodermic CTCL can be especially challenging. Histopathological analysis can reveal diagnosis of erythroderma in only 50% of patients (1). Immunohistochemical analysis of the cellular phenotype is

also, in most cases, of little help, since the erythrodermic CTCL is a malignancy of mature T-cells, and often there is no detectable loss of T-cell antigens (2).

Molecular genetic studies, based on the T-cell receptor-γ (TCR-γ) gene rearrangement analysis, became the standard in diagnosis of systemic and cutaneous lymphomas and leukemias. TCR gene rearrangement analysis detects T-cell clonality in cellular infiltrates. Detection of the dominant clone in skin infiltrate (monoclonal pattern) reflects the fact that the majority of T-cells in the lymphocyte infiltrates in patient's skin, belongs to the same clone, i.e. derives from the single cell. Rearrangement analysis of the γ chain of the TCR gene is most frequently used, since it is rearranged in all T-cells, irrespective of the final T-cell phenotype (αβ or γδ) (3).

T-cell clonality detection proved to be very useful in early diagnosis of mycosis fungoides, especially when the clinical and histopathological features are non-diagnostic (4-6). In addition, the detection of the dominant clone in the peripheral blood was found to be an independent prognostic factor in patients with mycosis fungoides (7). It is, also, an established criterion for the diagnosis of Sezary syndrome and erythrodermic cutaneous T-cell lymphoma (4, 8). Most of the available studies on TCR gene rearrangement analysis, in skin samples of patients with erythroderma, included patients with Sezary syndrome, or pseudolymphoma (7-9). There are only few studies available on the role of TCR-γ gene rearrangement anaysis in patients with erythroderma of varying origin, and the way it can aid the etiologic diagnosis (10, 11).

In this study, a PCR-based TCR-γ gene rearrangement analysis was used for detection of clonality of T-cells in skin infiltrates and peripheral blood of patients with erythroderma of varying origin treated in two dermatologic refferal centres in Serbia from 2001 to 2008.

T-cell clonality analysis results were correlated with clinical data, histopathological results and follow-up data, in order to establish an etiologic diagnosis of the disease. The aim of the study was to examine if the T-cell clonality analysis can help in distinguishing inflammatory erythroderma and erythroderma due to the lymphoma.

Patients and methods

Patients: Twenty-four patients were enrolled in the study. The patients were treated in two refferal dermatologic centers in Serbia: Department of Dermatology, Military Medical Academy and Institute of Dermatovenereology, Clinical Centre of Serbia, from 2001 to 2008. The inclusion criteria were: 1) clinical manifestation of erythroderma; 2) clinical suspicion of lymphoma; 3) skin samples available for histopathological, immunohistochemical and molecular genetic testing. The patients with preexisting skin diseases were excluded from the study, as well as patients with drug-induced erythroderma, in whom the diagnosis was confirmed by acute onset of erythroderma after ingestion of drug and resolution of the erythroderma after drug withdrawal. Five patients presented with erythroderma and plaques and/or tumors corresponding to mycosis fungoides. Final diagnosis of erythroderma was made by correlating clinical, histopathological and immunohistochemical data, and in cases of erythroderma due to the lymphoma - according to the WHO-EORTC classification of cutaneous lymphomas (4). All patients underwent, routine investigations needed for evaluation of patients with erythroderma as follows (12, 13): sedimentation rate, CRP, CBC, DBC, routine biochemistry, urinalysis, liver enzymes, peripheral blood smear, peripheral blood lymphocyte immunophenotype analysis in selected cases, skin biopsy for histopathological analysis, immunohistochemistry and TCR-γ gene rearrangement analysis, abdominal ultrasound, chest radiography, CT scan of chest and abdomen as indicated, cytological analysis of lymph nodes and/or lymph nodes biopsy in cases with lymphadenopathy, bone marrow biopsy as indicated by consultant hematologist and testing for HIV infection.

Informed consent was obtained from patients enrolled in the study. There were 20 men and 4 women, aged 31-85 years (median 63 years). Median duration of follow up period was 2 years (1-8 years).

Samples

From 2001 to 2008, 24 skin and 20 peripheral blood samples were analyzed. The samples were taken at the time of diagnosis, or during the relapse of disease after therapy, with excisional or shave biopsy. Half of the samples were placed in 10% formaldehyde for further

histopathological analysis, and the other half were frozen and stored at -20 °C for DNA extraction and further DNA analysis. The blood samples were taken by venepuncture and used for DNA extraction and further analysis.

TCR-γ gene rearrangement analysis

DNA was isolated by phenol: chloroform/isoamyl alcohol extraction (14).

PCRs were performed in a 50 μL reaction mixture containing 400-600 ng of gDNA (Master Mix, Applied Biosystems). For amplification of the hypervariable region of rearranged TCR-γ chain gene, 0.2 μM/L of each oligonucleotide primer was used (Table 1.).

Results

Patients with confirmed cutaneous T-cell lymphoma

After the completion of all investigations needed for evaluation of patients with erythroderma, cutaneous T-cell lymphoma was diagnosed in 9 patients. Three patients fulfilled the criteria for Sezary syndrome, and 6 were diagnosed with erythrodermic mycosis fungoides.

Table 2. presents clinical data, histopathological analysis and TCR gene rearrangement analysis of skin samples of patients with confirmed cutaneous T-cell lymphoma (CTCL).

Table 1. Primer sequences for amplification of TCR-γ gene hypervariable region

Gene	Region	Nucleotide sequence
		5'-C TTC ACT CAG ATG TCA CCT ACA ACT CCA AGG TTG-3'
		5'-C TTC CTG AAG ATG ACG CCT CCA CCG CAA GGG ATG-3'
		5'-C TTC CTG GGA ATG ACT ACC ACA CCT CCA GCG GTT-3'
		5'-C TTC CTG ATG GTA ACT CCT ACA ACT CCA GGG TTG-3'
TCR-γ	*V-J*	5'-GGNA CTG CAG GAA GGC AAT GGC GCA TTC CG-3'
		5'-GGNA AAA CAG GAA AGG AAT CTG GCA TTC CG-3'
		5'-AAG TGT TGT TCC TAC GCC TTT-3'
		5'-AGT TAC TAT TCT CCT AGT CCC-3'
		5'-TGT AAT GAT GGA CTT TGT TCC-3'

The PCR protocol included 40 repeats of the basic cycle (94°C for 40 seconds, 56°C for 1 minute, 72°C for 1 minute). The uniformity of rearranged TCR-γ genes was analyzed on 10% polyacrylamide gel (PAGs) electrophoresis after staining with silver nitrate (15).

To ensure that DNA was amplifiable, all samples were amplified with commercial primers for the P53 exon 4. In order to avoid false–positive results, negative controls, containing no template DNA, were subjected to the same procedure. PCR products were considered to be monoclonal only if one or two discrete band within the expected size range (~200 bp) was observed on the gel after electrophoresis.

In two patients, pathohistological analysis of skin samples was non-specific: in one, chronic inflammatory lymphocyte infiltrate was accompanied by a prominent eosinophilic infiltration, and the other presented with features of subacute dermatitis.

Patients with erythroderma due to benign inflammatory dermatoses

In 12 patients, clinical features and histopathological analysis of the skin samples were consistent with inflammatory dermatoses: 4 presented with adult-onset atopic dermatitis, 3 were diagnosed with disseminated eczema, 3 with senile erythroderma (after exclusion of paraneoplastic disease), 1 with pityriasis rubra pilaris, and 1 with paraneoplastic erythroderma accompanied by low-grade B-cell leukemia (Table 3). In this

Table 2. TCR-γ gene rearrangement in the skin samples of patients with confirmed CTCL

No.	Age/ Gender	Clinical diagnosis	Histopathological diagnosis		TCR-γ gene rearrangement (skin)	Final diagnosis
			First (date)	*Diagnostic (date)*		
1	75/M	Paraneoplastic erythroderma. CTCL in obs.	Subacute dermatitis (2000)	CTCL (2001)	Monoclonal	Sezary syndrome
2	63/M	Paraneoplastic erythroderma. CTCL in obs.	CTCL (2003)	CTCL (2003)	Monoclonal	Erythrodermic mycosis fungoides
3	49/F	Mycosis fungoides in bs. Erythroderma.	Mycosis fungoides (2003)	Mycosis fungoides (2003)	Polyclonal	Erythrodermic mycosis fungoides
4	48/M	Paraneoplastic erythroderma. CTCL in obs.	CTCL (2003)	CTCL (2003)	Monoclonal	Erythrodermic mycosis fungoides
5	66/M	Mycosis fungoides in obs. Erythroderma.	Parapsoriasis en plaque (1999)	Mycosis fungoides (2001)	Monoclonal	Erythrodermic mycosis fungoides
6	62/M	Paraneoplastic erythroderma. CTCL in obs.	Mycosis fungoides (2001)	Mycosis fungoides (2001)	Monoclonal	Erythrodermic mycosis fungoides
7	57/M	Mycosis fungoides Erythroderma.	Mycosis fungoides (2001)	Mycosis fungoides (2001)	Monoclonal	Erythrodermic mycosis fungoides
8	46/F	Adult onset atopic dermatitis. CTCL in obs.	Subacute dermatitis (2006)	Chronic nonspecific dermatitis (2007)	Monoclonal	Sezary syndrome
9	31/M	Adult onset atopic dermatitis. CTCL in obs.	Chronic nonspecific dermatitis (2007)	Chronic nonspecific dermatitis (2008)	Monoclonal	Sezary syndrome

Obs. – Abbreviated from: Observed and expected

patient, monoclonal pattern was found in the skin, blood and bone marrow in B-cell receptor (immunoglobulin gene) rearrangement analysis, while the polyclonal pattern was found in the skin sample for TCR-γ gene rearrangement analysis.

T-cell clonality analysis in all our patients with inflammatory dermatoses revealed a polyclonal pattern.

Patients with idiopathic erythroderma and clinical suspicion of lymphoma

In three patients, in spite of vigorous researches, the true nature of presenting erythroderma was not elucidated (Table 4).

Two patients presented with generalized redness and lymphadenopathy, diffuse alopecia, fever and prominent weight loss during the previous months.

Table 3. TCR-γ gene rearrangement in patients with inflammatory dermatoses

No.	Age/ Gender	Clinical diagnosis	Histopathological diagnosis	TCR-γ gene rearrangement (skin)	Final diagnosis
1	80/M	Disseminated eczema. CTCL in obs.	Eczematoid dermatitis	Polyclonal	Disseminated eczema
2	85/M	Paraneoplastic erythroderma. CTCL in obs.	Superficial perivascular dermatitis	Polyclonal	Senile erythroderma
3	71/F	Adult onset atopic dermatitis. CTCL in obs.	Chronic spongiform dermatitis	Polyclonal	Adult onset atopic dermatitis
4	63/M	Pityriasis rubra pilaris in obs. CTCL in obs.	Pityriasis rubra pilaris	Polyclonal	Pityriasis rubra pilaris
5	59/M	Adult onset atopic dermatitis. CTCL in obs.	Chronic spongiform dermatitis	Polyclonal	Adult onset atopic dermatitis
6	73/F	Adult onset atopic dermatitis. CTCL in obs.	Chronic spongiform dermatitis	Polyclonal	Adult onset atopic dermatitis
7	61/M	Adult onset atopic dermatitis. CTCL in obs.	Acute spongiform dermatitis	Polyclonal	Adult onset atopic dermatitis
8	82/M	Erythrodermia paraneoplastica seu senilis. CTCL in obs.	Superficial perivascular dermatitis	Polyclonal	Senile erythroderma
9	64/M	Paraneoplastic erythroderma. CTCL in obs.	Superficial perivascular dermatitis	Polyclonal	Paraneoplastic Erythrodermia (low-grade B-cell leukemia)
10	68/M	Disseminated eczema. CTCL in obs.	Chronic spongiform dermatitis	Polyclonal	Disseminated eczema
11	54/M	Disseminated eczema. CTCL in obs.	Chronic spongiform dermatitis	Polyclonal	Disseminated eczema
12	83/M	Paraneoplastic erythroderma. CTCL in obs.	Chronic dermatitis with prominent eosinophilic infiltration	Polyclonal	Senile erythroderma

Obs. – Abbreviated from: Observed and expected

Protracted erythroderma was resistant to corticosteroid therapy, which only temporarily and in medium doses controlled the signs and symptoms of the disease. In the first patient, the disease was present for 8 years, with partial remissions and exacerbations, and repeated histopathological analyses revealed chronic dermatitis, with spongiform features. However, by repeated TCR-γ gene rearrangement analysis of the skin and peripheral blood samples, at the last control, detected a dominant clone, and immunophenotype analysis of peripheral blood lymphocytes detected significant predominance of CD4+ T-cells, with increased CD4/CD8 index. Since atypical lymphocytes were not detected by histopathological analysis and in peripheral blood smears, these criteria were considered insufficient for diagnosis of Sezary syndrome. Acitretin was introduced, with slow tapering from systemic corticosteroids, and the patient should be further followed-up for final diagnosis. In the second patient erythroderma lasted for 2-years and it was accompanied by fever, severe pruritus and lichenification, diffuse alopecia, 10 kg weight loss in 2 months, and axillary and inguinal lymphadenopathy (up to 3 cm in diameter and in packages). Although clinical features were highly suspicious for lymphoma, histopathological analysis of the skin and lymph node samples, peripheral blood smears and immunophenotype analysis, radiographic studies and searches for neoplasia did not reveal any signs of a malignant (hematologic or other) disease. However, repeated TCR-γ gene rearrangement analysis, detected a dominant clone in the skin and peripheral blood sample. The patient was treated with systemic corticosteroids and should be followed-up. In cases with idiopathic erythroderma, cutaneous T-cell lymphoma, atopic dermatitis and drug reactions were found to be the most common causes, during the follow up, in one study, and in the other, CTCL was the most common diagnosis during the follow-up of patients with idiopathic erythroderma (11, 16).

Table 4. TCR-γ gene rearrangement in patients with idiopathic erythroderma

No.	Age/ Gender	Clinical diagnosis	Histopathological diagnosis	TCR-γ gene rearrangement (skin)	Final diagnosis
1	65/M	Paraneoplastic erythroderma. CTCL in obs.	Chronic dermatitis	Monoclonal	Idiopathic erythroderma. Sezary syndrome in obs.
2	63/M	Paraneoplastic erythroderma. CTCL in obs.	Superficial perivascular dermatitis	Monoclonal	Idiopathic erythroderma. CTCL in obs.
3	59/M	Mycosis fungoides in obs Erythroderma.	Chronic spongiform dermatitis Diffuse T-cell hyperplasia	Monoclonal	Erythroderma sec. CTCL in obs.

Obs. – Abbreviated from: Observed and expected

The third patient was admitted to our Department with erythroderma and tumorous lesions on the chest, arms and legs. Clinical diagnosis of erythrodermic mycosis fungoides was highly probable. However, histopathological analysis of one skin sample was consistent with spongiform dermatitis while in the tumorous lesion, diffuse T-cell hyperplasia was found, but with no signs of lymphocyte atypia and loss of T-cell markers (CD5, CD7). Nevertheless, dermopathic lymphadenopathy was found in lymph node biopsy, CT scan of the chest and abdomen did

not reveal any signs of systemic lymphadnopathy and hepatospleno-megaly. Bone marrow histopathological analysis was also normal. However, TCR-γ gene rearrangement analysis detected a dominant clone in the skin and polyclonal pattern in peripheral blood. Infectious etiology of the disease, and presence of other malignant disease were also excluded. Since there were insufficient criteria for diagnosis of cutaneous T-cell lymphoma, therapy with acitretin and systemic corticosteroids and close follow-up was indicated.

TCR-γ gene rearrangement analysis in peripheral blood samples

In patients with erythroderma and confirmed cutaneous T-cell lymphoma, a dominant T-cell clone was detected in peripheral blood of 5 patients. In one patient polyclonal pattern was found in peripheral blood, and in three the analysis was not performed. In patients with inflammatory dermatoses, a dominant clone was found in peripheral blood in one patient with senile erythroderma (a dominant clone was not found in the skin), and in one patient with erythroderma accompanying low-grade B-cell leukemia. Of 3 patients with idiopathic erythroderma, a dominant clone was found in 2 patients: in both in the skin and in peripheral blood.

Discussion

The incidence of erythroderma (or exfoliative dermatitis) was estimated to be 35 per 100.000 of dermatotological outpatients in one study, but there are no data on overall incidence (17). The causative factors are previous dermatoses, drug reactions, malignancy, infections and idiopathic erythroderma. The 4 most common causes are: adult atopic dermatitis, drug reactions, cutaneous T-cell lymphoma (CTCL) and paraneoplastic erythroderma (12, 13, 16, 18, 19). In our study, drug reactions and previous dermatoses were excluded, and there were 9 patients with erythrodermic CTCL, 3 with idiopathic erythroderma (and probable prelymphomatous eruption), 8 with adult onset atopic dermatitis and disseminated eczema, 2 patients with senile erythroderma, 1 patient with pityriasis rubra pilaris, and 1 with paraneoplastic erythroderma. This is in concordance with other studies of patients with erythroderma, where these entities are the most common (apart from previous dermatoses and drug eruptions). In our patients, male to female ratio was 5:1, and the mean age of presentation 63 years, similar to other studies (12).

Histopathological analysis of skin samples can be a diagnostic criterion in up to 50% of patients, but usually after repeated biopsies (1). In our patients, histopathological analysis was diagnostic in 13 of 24 patients (54.1%), which corresponds with the data from other studies. In patients with inflammatory dermatoses, specific features were found in 8 of 12 patients (7 with eczema and 1 with pityriasis rubra pilaris) while in the other 4, nonspecific perivascular superifical dermatitis with varying presence of eosinophils was found. In patients with erythrodermic CTCL, the first pathological analysis was diagnostic in 5 of 9 patients, and in repeated biopsies in 7 of 9 patients. In two patients, histopathological features of skin sample analysis remained non-specific, but patients fullfilled criteria for Sezary syndrome: presence of Sezary cells $\geq 1000/mm^3$ in peripheral blood, immunophenotype abnormalities with loss of T-cell antigen markers, and dominant T-cell clone in peripheral blood (4, 8).

In patients with erythrodermic CTCL, TCR-γ gene rearrangement analysis detected a dominant clone in 8 of 9 patients (88.8%). In other studies, a dominant clone was found in erythrodermic CTCL in up to 83% of patients, which is in concordance with our results (7, 10, 20). In 1 patient with erythrodermic mycosis fungoides, a dominant clone was not detected. In the late stages of the disease, there is a possibility of TCR gene deletion only during malignant transformation (21). Also, this can be a false-negative result, due to poor sampling, i.e. taking the skin sample with small number of malignant T-cells, and there is a possibility that primers used in this study did not cover all possible TCR-γ gene rearrangements (5, 21, 22).

In contrast, in the group of erythroderma due to inflammatory dermatoses, a dominant clone was not detected in any of the samples. This clearly demonstrates that in patients with erythroderma, TCR-γ gene rearrangement analysis is a useful adjunct to diagnosis, since it can differentiate between polyclonal (e.g benign), and monoclonal (e.g lymphomatous) T-cell population in the cellular infiltrate. Cherny et al., in their series of 16 patients with erythroderma

found that finding of a dominant clone was specific for lymphomatous erythroderma (11). Cordel and co-authors found a dominant clone in the skin samples of 7 patients with suspected lymphoma and in none of 16 patients with probable inflammatory dermatoses (as determined by the dermatopathologist) (10). TCR-γ gene rearrangement analysis mainly increased the sensitivity of diagnosis of erythrodermic mycosis fungoides in the other study: the sensitivity was 62% with histopathological analysis, and 87% with a combined histopathological analysis and T-cell clonality analysis (9).

However, it should be noted that the finding of a dominant clone does not imply malignancy in every case. There is a possibility of „false-positive" results, in cases where more than 1% of reactive benign lymphocytes of the same clone is present in the cutaneous infiltrate. In these cases, monoclonality is detected, but is not a sign of malignancy.

In our study, three patients were present with so-called „clonal dermatitis", i.e. finding of non-specific dermatitis by histopathological analysis and a dominant clone by PCR. Patients with «clonal dermatitis» require close follow-up and repeated biopsies, because of a possible underlying lymphoproliferative disorder, not recognized at the beginning of signs and symptoms of the disease (23).

One patient with „clonal dermatitis", presented with two criteria for Sezary syndrome (SS). The International Society for Cutaneous Lymphomas (ISCL) recommendation criteria for the diagnosis of SS include one or more of the following: an absolute Sezary cell count of at least 1000 cells/mm^3; demonstration of immunophenotypical abnormalities (an expanded CD4$^+$ T-cell population resulting in a CD4/CD8 ratio over 10, loss of any or all of the T-cell antigens CD2, CD3, CD4, and CD5, or both); or demonstration of a T-cell clone in the peripheral blood by molecular or cytogenetic methods (8). In addition, if the skin and lymph node studies are showing non-specific features and are not diagnostic, additional evidence of malignancy are required for final diagnosis of Sezary syndrome, such as: presence of large Sezary cells, presence of the same dominant clone in the skin and blood, or aberrant expression of T-cell markers (8). Since the clinical validity of these criteria has not yet been established in a large study, WHO-EORTC believe that finding of a dominant T-cell clone in the peripheral blood (preferably the same as in the skin) together with one of the cytomorphologic or immunophenotypic criteria, should be a minimum for diagnosis of Sezary syndrome. In our patient, two ot the minimum criteria were met, and the patient was closely followed-up to confirm these findings in repeated analyses. In the meanwhile, therapy with acitretin and slowly tapered systemic corticosteroids was introduced, with satisfying results. In the second patient, a dominant clone in the skin and the peripheral blood was found, but the cytomorphologic and immunophenotypic criteria were not detected, so the patient was closely followed-up. In the third patient with "clonal dermatitis", clinical features with erythroderma and tumorous lesions pointed out to the erythrodermic mycosis fungoides, but repeated histopathological analyses were non-diagnostic. A dominant clone was repeatedly found in the skin (not in the blood), and in one sample, T-cell diffuse hyperplasia was found but with no signs of lymphocyte atypia. In this case, acitretin and systemic corticosteroids were introduced with good effects, and close follow-up was also necessary for final diagnosis.

On the other hand, in two patients with benign inflammatory dermatoses (aged 85 and 65), a dominant clone was found in peripheral blood samples, but not in the skin. The significance of the isolated finding of peripheral blood monoclonality is not yet established. It can be found in healthy subjects older then 80 years, and also in patients with certain autoimmune disorders (24, 25). In this study, a dominant clone was found in one patient aged 85 years, with senile erythroderma, and it was not found in the skin sample, but non-specfic dermatitis was found by histopathological analysis. Search for internal malignancy, bone marrow biopsy, and lymph node biopsy, among other investiagations, did not reveal any signs of malignant disease, and the patient was diagnosed to have senile erythroderma. Senile erythroderma is most commonly manifested by previous eczema or atopic dermatitis, with persistent course and nonspecific features by histopathological analysis, elevated IgE and LDH and variable presence of eosinophils in the skin biopsy (16). In the other patient, aged 65, a dominant T-cell clone was found only in the peripheral blood, while a dominant B-cell clone was found in the bone

marrow, peripheral blood and skin samples. This patient was diagnosed to have low-grade B-cell leukemia (the bone marrow histopathological analysis was diagnostic), and the finding of a dominant T-cell clone and erythroderma, can point to possible false-positive result due to existence of more than 1% of reactive T-cells, which were activated by malignant B-cell clone during the course of the disease. There is a possibility that these T-cells were responsible for erythroderma, which is not an usual manifestation of low-grade B-cell leukemia. Less probable is a possibility of composite B- and T-cell lymphoma (yet not fully developed) which is very rare (26).

In the group of patients with erythrodermic CTCL, a dominant clone was found in 5 of 6 patients in whom analysis was performed. The finding of the same dominant clone in peripheral blood and in the skin sample (and/or lymph node) may be a disease manifestation, and in this case represents an independent factor of poor prognosis (7, 27, 28). In our previous study, presence of a dominant clone, both in the skin and peripheral blood of patients with mycosis fungoides, was detected in 7/16 (43.8%) of patients with end-stage disease, while in patients with early-stage disease, it was present in 1/7 patients (14.2%) (29). In 3/4 (75%) of patients with disease, a remission of polyclonal pattern was detected, while in patients with stable disease and disease progression, polyclonal pattern in skin/blood was present in only 3 of 14 patients (21.4%). Also, a trend toward shorter time to progression was found in patients with a dominant clone, both in the skin and peripheral blood, in comparison to patients in whom a dominant clone was not found (29). Although these differences were not statistically signficant, they may have a prognostic importance of a dominant clone in the skin/peripheral blood, which was found in other studies (7, 27, 28).

In conclusion, TCR-γ gene rearrangement analysis was found to be useful in diagnosis of patients with erythroderma. Findings of a dominant clone in the skin and peripheral blood should be correlated with the clinical, histopathological and immunophenotypical features, for the final diagnosis, and proper treatment of patients with erythroderma.

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Dijagnostički značaj analize preuređenja gena gama lanca T-ćelijskog receptora kod pacijenata sa eritrodermijom

Sažetak:

Uvod: Etiološka dijagnoza eritrodermije, predstavlja veliki izazov, s obzirom da kliničke manifestacije, histopatološka analiza i imunofenotipizacija ćelijskog infiltrata kod mnogih bolesnika nije dovoljna za diferencijalnu dijagnozu inflamatorne eritrodermije i eritrodermijskog kutanog T-ćelijskog limfoma. U ovoj studiji, klonalnost T–limfocita ispitana je analizom gena za γ-lanac T-ćelijskog receptora u uzorcima kože i periferne krvi pacijenata sa eritrodermijom različitog porekla, radi ispitivanja značajnosti ove analize u dijagnostici eritrodermije.

Metodi: Uzorci kože i periferne krvi su uzeti kod 24 pacijenata sa eritrodermijom, lečenih od 2001 do 2008 u Klinici za kožne i polne bolesti Vojnomedicinske akademije i Instituta za dermatovenerologiju Kliničkog centra Srbije. Multipleks PCR korišćen je za analizu rearanžmana gena za γ–lanac T-ćelijskog receptora.

Rezultati: 20 muškaraca i 4 žene bilo je uključeno u ispitivanje. Dijagnoza eritrodermijskog primarnog kutanog T-ćelijskog limfoma postavljena je kod 9 bolesnika, dok je 12 pacijenata dijagnostikovana neka od inflamatornih dermatoza. U grupi pacijenata sa eritrodermijskim kutanim T-ćelijskim limfomom, kod 8 od 9 pacijenata (88.8%) detektovan je dominantni klon u uzorku kože, i u 5 od 6 uzoraka periferne krvi. U grupi inflamatornih dermatoza, dominantni klon nije nađen ni u jednom uzoraku kože, dok u perifernoj krvi nađen u 2 od 12 uzoraka. Kod 3 pacijenta uprkos iscrpnim ispitivanjima uzrok eritroderme nije utvrđen, a u uzorcima kože je viđen tzv. «klonski dermatitis», te su savetovane česte kontrole i ponavljane biopsije, zbog moguće dijagnoze limfoproliferativnog oboljenja.

Zaključak: Detekcija T-ćelijske klonalnosti analizom gena za γ-lanac T-ćelijskog receptora značajno doprinosi diferencijalnoj dijagnozi inflamatornih eritrodermija i eritrodermijskog primarnog kutanog T-ćelijskog limfoma.