https://doi.org/10.17116/engklinderma201817172-75

Experience in application of the confocal laser scanning microscopy of the skin is diagnosis of primary cutaneous diffuse large B-cell lymphoma

N.V. KUNGUROV, I.A. KUKLIN*, M.M. KOKHAN, N.V. ZILBERBERG, E.P. TOPYCHKANOVA, I.D. KUZNETSOV

Ural Research Institute of Dermatovenerology and Immunopathology, Ekaterinburg, Russia, 620076

The article provides a brief literature review on the incidence, classification, clinical manifestations, as well as the modern methods of diagnosis and treatment for primary cutaneous diffuse large B-cell lymphomas. The authors report a case of preliminary diagnosis of the disease using confocal laser scanning microscopy of the skin at the pre-biopsy stage followed by diagnosis verification using pathomorphological and immunohistochemical studies of skin bioptate.

Keywords: confocal laser scanning microscopy of the skin, primary cutaneous diffuse large B-cell lymphoma, clinical presentation, diagnosis, treatment.

Primary skin lymphomas (PSLs) form a heterogeneous group of diseases caused by cutaneous neoplastic proliferation of clones of T-lymphocytes, NK-cells, or B-lymphocytes affine to skin tissue. According to the literature, the incidence of primary cutaneous lymphomas in the world varies from 0.3 to 1.18 cases per 100 thousand population and continues to increase. Primary cutaneous B-cell lymphomas account for 10 to 30% of all PSLs and are usually characterized by relatively uniform clinical course and favorable prognosis [1—3].

According to the WHO-EORTC classification, B-cell lymphomai of the skin are classified into the following five types:

- primary cutaneous B-cell lymphoma of the marginal zone;
- primary cutaneous follicle center cell lymphoma;
- primary cutaneous diffuse large B-cell lymphoma, leg type;
- primary cutaneous diffuse large B-cell lymphoma, other type;
- intravascular large B-cell lymphoma [1,2,4,5].

Primary cutaneous diffuse large B-cell lymphoma, leg type, account for about 5—10% of all cutaneous B-cell lymphomas. This form of B-cell lymphoma occurs mainly in older women (median, 78 years old) and presents with fast-growing nodes and plaques located on the skin of the lower extremities. Infiltration of the dermis with centroblasts, immunoblasts, and, to a lesser extent, centrocytes is observed. Tumor cells diffusely infiltrate the dermis, which is accompanied by replacement of normal tissues and obliteration of the appendages. Infiltrate can penetrate the subcutaneous tissue. The epidermis is usually intact and separated by a zone of intact collagen (Grenz zone). There is mild reactive infiltration and stromal reaction. Tumor cells express CD20+, CD79a+, bcl2+, MUM-1/IRF4+, and FOX-P1+. Five-year survival averages 55% [6—8].

Another type of primary cutaneous diffuse large B-cell lymphoma is similar to primary cutaneous diffuse large B-cell lymphoma, leg type, in many pathomorphologic characteristics. Its clinical presentation is characterized by solitary nodes located in other areas of the skin, usually on the head and neck. Tumor infiltrate expresses CD20+, CD79a+ [4,7]

The diagnostic process involves a comprehensive evaluation of the clinical presentation and analysis of the results of pathomorphological and immunohistochemical studies of skin bioptates indicative of the presence of a malignant lymphoid proliferation in the skin. However, skin biopsy is not always possible due to several reasons, including ethical (when there is no voluntary informed consent of the patient for manipulations), or when the tumor is located in the areas that are cosmetically significant and problematic for the patient.

In this situation, confocal laser scanning microscopy of the skin (CLSM), a high-tech non-invasive *in vivo* method for examination of skin pathomorphology, is particularly valuable.

CLSM is a unique diagnostic technique that enables multiple examinations of various lesions in the same patient with a resolution close to that of conventional light microscopy without disturbing skin integrity.

The advantages of this method are as follows:

- high speed of the test compared to conventional pathomorphological examination;
- possibility of examination of epidermis and dermis in 5-μm-thick layers
- analysis of the cellular composition of the epidermis and upper layers of dermis;
- evaluation of papillary dermis vascularization, including the diameter of the capillaries;
- Evaluation of the area and the level of tumor infiltration.

The available international and Russian literature dealing with the application of CLSM in dermatology describes cytoarchitectonics of intact skin and reports the results of the studies of pathomorphological changes associated with skin photoaging, tumors (actinic keratosis,

*e-mail: kuklin71@mail.ru

basal cell carcinoma, melanoma), inflammatory and infectious diseases (psoriasis, rosacea, onychomycosis, tinea, allergic contact dermatitis) [9—13].

No research into pathomorphology of epidermis and dermis in patients with primary cutaneous lymphomas using CLSM were reported in the Russian literature, while the existing international literature provides only scarce descriptive data on this issue. However, these data show high diagnostic value of the method and correlation between the results and the data of histological examination of skin biopsy samples [14—17].

Diagnostic capabilities of CLSM in primary cutaneous lymphomas are being studies at the clinic of the Ural Research Institute of Dermatovenerology and Immunopathology. We exemplify the use of CLSM method in the diagnosis of primary cutaneous diffuse large B-cell lymphoma by our own case report.

Patient Z., born in 1949, applied to the clinic of the Ural Research Institute of Dermatovenerology and Immunopathology with complaints of scalp tumor painful on palpation.

Anamnesis morbi. The patient believes that she is sick since 2 years ago, when she first noticed a small element on the scalp, which was not accompanied by subjective sensations. During the 1st month of the disease, the patient visited dermatologist at the place of residence, where she was diagnosed with "localized scleroderma". No improvement was observed after the course of topical therapy (hydrocortisone ointment TID for 14 days). According to the patient, the tumor gradually increased in size and there was palpatory tenderness. Since therapy was ineffective and skin process worsened, the patient was sent to the Ural Research Institute of Dermatovenerology and Immunopathology for further diagnosis.

Life history, allergic anamnesis, and occupational history are unremarkable. Family history of skin diseases and cancer pathology is not burdened.

Objective data. The patient is of average height, regular physique, normotrophic. Lungs: vesicular breathing, no wheezing. Clear rhythmic heart tones, blood pressure 130/86 mm Hg, heart rate of 70 bpm. The abdomen is soft and painless on palpation, the liver and spleen are not enlarged. There are palpable oval and round submandibular lymph nodes; they are elastic, painless, mobile, up to 1 cm in diameter. Physiological functions are normal.

Status localis. Intact skin areas have physiological color, normal turgor and humidity. Visible mucous membranes are moist and have physiological color. The skin process is represented by an irregular dense pink-red tumor of 4.5 x 4 cm in diameter localized in the right occipital region of the scalp. The lesion is infiltrated and rises above the skin surface by 0.5 cm, has an uneven and nodular surface with partially preserved hair follicles and hair growth (Fig. 1). Hair and nail plates are intact. Dermographism is red, fast.

Laboratory data. Complete blood count: hemoglobin 131 g/l, erythrocytes $4.21x10^{12}$/l, leukocytes $5.5x10^{9}$/l, neutrophils $2.8x10^{9}$/l, eosinophils $0.3x10^{9}$/l, lymphocytes $2.1x10^{9}$/l, monocytes $0.3x10^{9}$/l, ESR 5 mm/h. Clinical urine analysis and immunogram are without abnormalities. Biochemical hepatogram showed increase in cholesterol levels to 6.53 mmol/l. Serological tests for *Treponema pallidum* are negative. No antibodies to HIV, hepatitis B, and C were detected.

Skin cancer was suspected in this patient at the consultation of dermatologists at the clinic of the Ural Research Institute of Dermatovenerology and Immunopathology, and therefore we recommended the incisional skin biopsy of the lesion. However, since the patient flatly refused skin biopsy and did not signed a voluntary

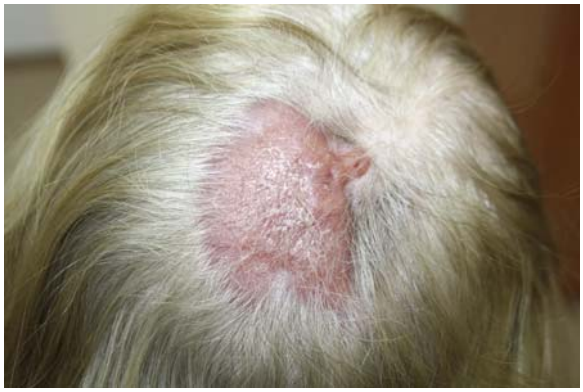

Fig. 1. **Patient Z. Tumor in the occipital region of the scalp with a partially preserved hair growth.**

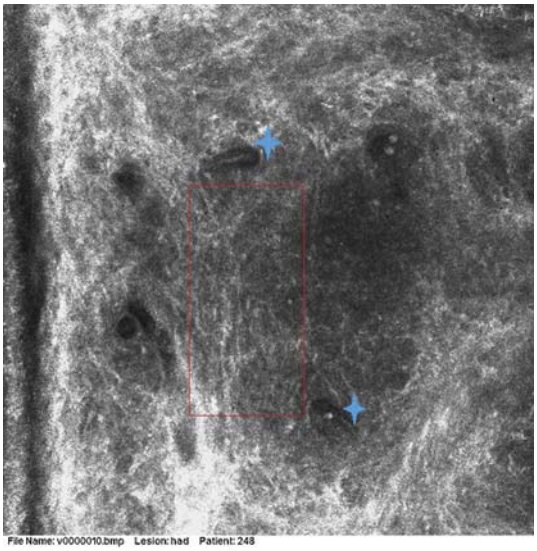

Fig. 2. **Patient Z. CLSM of the dermal-epidermal junction of the skin lesion.**

There is abnormal architectonics of the epidermis and dermis ("flow" symptom), as evidenced by focal axial extension of keratinocytes (red rectangle); transversely dilated capillaries with increased blood flow (blue stars).

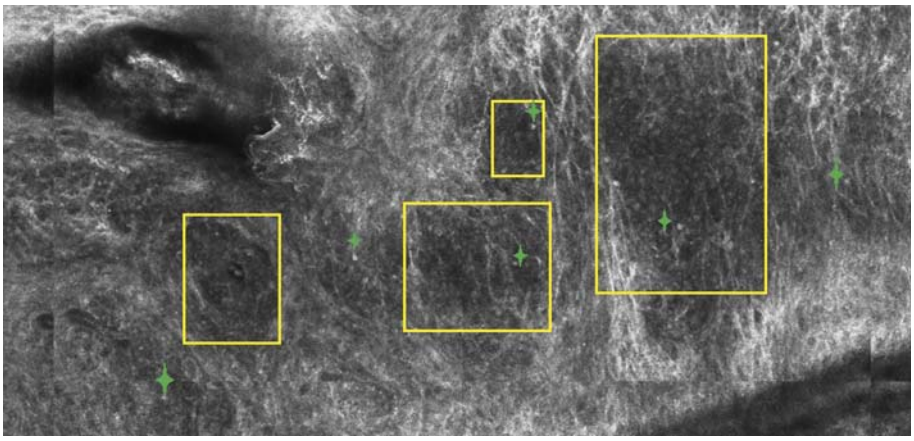

Fig. 3. **Patient Z. CLSM of the skin lesion (94.5 mm in depth).**
Rounded atypical cells, presumably lymphocytes (green stars), and multiple tumor infiltrates, destroying the stromal structure of the dermis (yellow rectangles) are observed.

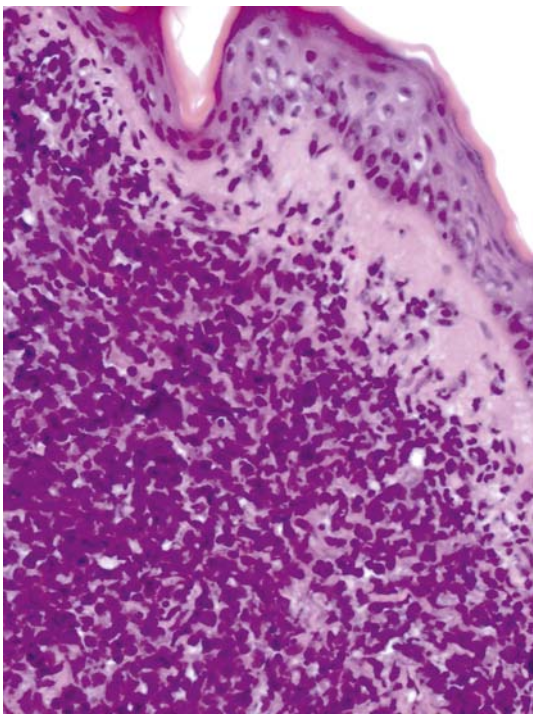

Fig. 4. **There is diffuse infiltration of the dermis consisting of lymphoid cells, mostly large with variable shape of nuclei and high mitotic activity.**
There is no significant epidermotropizm (magnification x 400).

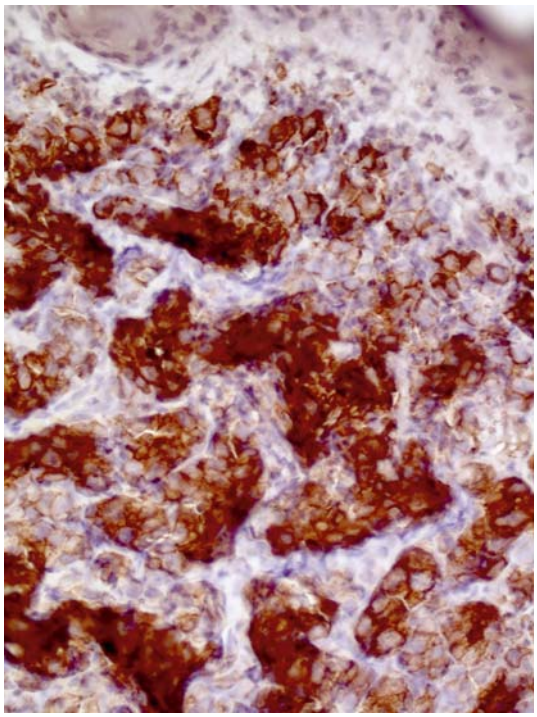

Fig. 5. **Numerous large CD20+ lymphocytes in the dermal infiltrate.**
Intact epidermis, the presence of the Grenz zone.

informed consent to this manipulation, we carried out *in vivo* CLSM on a Vivascope 1500 microscope (Germany).

CLSM examination of patient Z. showed signs of tumor, including focal axial extension of keratinocytes, resulting in abnormal architectonic of the epidermis and dermis ("flow" symptom); dilated capillaries with enhanced blood flow; numerous rounded atypical cells, presumably lymphocytes; multiple tumor infiltrates, destroying the stromal structure of the dermis (Fig. 2, 3). CLSM data allowed us to persuade the patient that skin biopsy is required for final verification of the diagnosis.

The results of a pathomorphological examination of a skin biopsy sample. There are no significant abnormalities of the epidermis. In the dermis, there is diffuse dense infiltrate without signs of epidermotropism formed by large

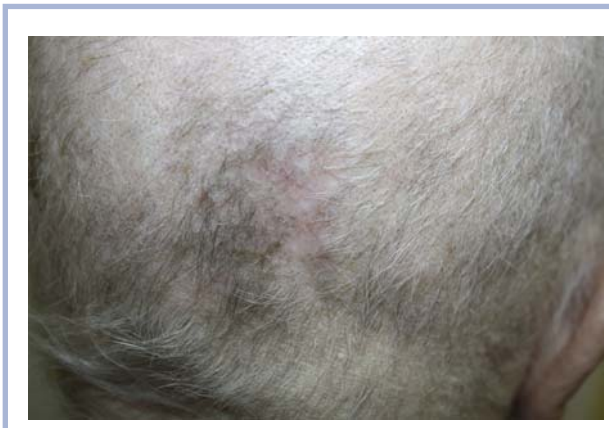

Fig. 6. **The condition of patient Z. after two courses of chemotherapy.**

lymphoid cells with amphophilic cytoplasm, round and oval cores. The high level of mitosis and apoptosis of the proliferate cells was observed (Fig. 4).

Immunohistochemical examination of a skin biopsy sample: tumor cells diffusely express CD20 (L26) and do not express CD3 (SP7), Bcl-2 (100/D5), CD10 (56C6), CD30 (Ber-H2). There is minimum number of reactive T-lymphocytes (CD3+, Bcl-2+, CD20-). Ki67 index (SP6) is about 90% (Fig. 5). Conclusion: histology and immunophenotype of the tumor correspond to primary cutaneous diffuse large B-cell lymphoma, Bcl-2 negative.

On the basis of medical history, clinical and pathological data, and the results of immunohistochemical studies of patient's skin bioptate, the following final nosological diagnosis was established: "Primary cutaneous diffuse large B-cell lymphoma, other type, Bcl-2 negative, IA (Ann Arbor)". The patient was sent for treatment to a hematologist.

Two courses of polychemotherapy at the city hematological center according to R-CHOP protocol resulted in complete tumor regression (Fig. 6). The patient is followed by a hematologist at the place of residence.

Discussion

The reported clinical case demonstrates that CLSM can be successfully used as a preliminary diagnostic technique at the pre-biopsy stage, since it enables detection of pathomorphological signs of the epidermal and dermal cytoarchitectonics characteristic of primary cutaneous lymphoma without disturbance of skin integrity and, in certain clinical situations, justifies the need for clinical and laboratory study using histological and immunohistochemical examination of a skin biopsy sample.

ЛИТЕРАТУРА/REFERENCES

1. Haverkos B, Tyler K, Gru AA. Primary cutaneous B-cell lymphoma: managment and patterns of recurrence at the multimodality cutaneous lymphoma Clinic of The Ohio State University. *The Oncologist*. 2015;20:1161-1166.
2. Lima M. Cutaneous primary B-cell lymphomas: from diagnosis to treatment. *An Bras Dermatol*. 2015;5(90):687-706.
3. Малишевская Н.П., Кохан М.М., Соколова А.В., и др. *Дерматоонкология (злокачественные новообразования кожи, первичные кожные лимфомы): Атлас*. Под ред. Кунгурова Н.В. Екатеринбург: Университет. 2016;168. [Malishevskaya NP, Kokhan MM, Sokolova AV, et al. *Dermatooncology (malignant neoplasms of skin, primary skin lymphomas): Atlas*. Ed. NV. Kungurov. Ekaterinburg. 2016;168. (In Russ.)].
4. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood*. 2005;105(10):3768-3785.
5. Кубанова А.А. (ред.) *Федеральные клинические рекомендации. Дерматовенерология 2015: Болезни кожи. Инфекции, передаваемые половым путем*. 5-е изд., перераб. и доп. М. 2016;768. [Kubanova AA. (ed.). *Federal clinical guidelines. Dermatovenereology 2015: Diseases of the skin. Sexually transmitted infections*. 5th ed. revised and enlarged edition. M. 2016;768. (In Russ.)].
6. Виноградова Ю.Е., Потекаев Н.С., Виноградов Д.Л. Лимфомы кожи: диагностика и лечение. *Практическая медицина*. 2014;176. [Vinogradova YuE, Potekaev NS, Vinogradov DL. Lymphomas of the skin: diagnostics and treatment. *Practical medicine*. 2014;176. (In Russ.)].
7. Молочков А.В., Ковригина А.М., Кильдюшевский А.В., Караулов А.В. *Лимфома кожи*. М. 2012;184. [Molochkov AV, Kovrigina AM, Kildyushevskiy AV, Karaulov AV. *Lymphoma of the skin*. M. 2012;184. (In Russ.)].
8. *Дерматоонкология*. Под ред. Галил-Оглы Г.А., Молочкова В.А., Сергеева Ю.В. М. 2005;872. [*Dermatooncology*. Eds. Galil-Ogly GA, Molochkov VA, Sergeev YuV. M. 2005;872. (In Russ.)].
9. Лукашева Н.Н., Ткаченко С.Б., Потекаев Н.Н., и др. Прижизненная отражательная конфокальная лазерная сканирующая микроскопия: история создания, принцип работы, возможности применения в дерматологии. *Клиническая дерматология и венерология*. 2008;5:10-15. [Lukasheva NN, Tkachenko SB, Potekaev NN, et al. In vivo reflectance confocal microscopy: history, working principle, use in dermatology. *Clin Dermatol Venereol*. 2008;5:10-15. (In Russ.)].
10. Волков А.И., Фриго Н.В., Знаменская Л.Ф., Катунина О.Р. Применение конфокальной лазерной сканирующей микроскопии в биологии и медицине. *Вестник дерматологии и венерологии*. 2014;1:17-24. [Volkov AI, Frigo NV, Znamenskaya LF, Katunina OR. Use of reflectance confocal microscopy in biology and medicine. *Vestnik dermatol venereol*. 2014;1:17-24. (In Russ.)].
11. Кубанова А.А., Чикин В.В., Штиршнайдер Ю.Ю., Катунина О.Р. Конфокальная лазерная сканирующая микроскопия *in vivo* в диагностике меланоцитарных новообразований кожи. *Вестник дерматологии и венерологии*. 2014;3:85-94. [Kubanova AA, Chikin VV, Shtirshneider YY, Katunina OR. Reflectance confocal microscopy in vivo in the diagnostics of melanocytic skin tumors. *Vestnik dermatol venereol*. 2014;3:85-94. (In Russ.)].
12. Кубанова А.А., Кубанов А.А., Смольянникова В.А., и др. Диагностическая значимость метода конфокальной лазерной сканирующей *in vivo* микроскопии. *Вестник дерматологии и венерологии*. 2015;3:68-74. [Kubanova AA, Kubanov AA, Smolyannikova VA, et al. Diagnostic value of the method of reflectance confocal microscopy in vivo microscopy. *Vestnik dermatol venereol*. 2015;3:68-74. (In Russ.)].
13. Снарская Е.С., Ткаченко С.Б., Кузнецова Е.В. Конфокальная лазерная сканирующая микроскопия в оценке стадий дерматогелиоза. *Российский журнал кожных и венерических болезней*. 2016;4:220-226. [Snarskaya ES, Tkachenko SB, Kuznetsova EV. Reflectance confocal microscopy in the evaluation of the stages of dermatoheliosis. *Rus J skin vener bol*. 2016;4:220-226. (In Russ.)]. https://doi.org/10.18821/1560-9588-2016-19-4-220-226
14. Agero AL, Gill M, Ardigo M, et al. In vivo reflectance confocal microscopy of mycosis fungoides: a preliminary study. *J Am Acad Dermatol*. 2007;57(3):435-441.
15. Gonzalez S, Gill M, Halpern AC. *Reflectance confocal microscopy of cutaneous tumors*. Informa UK Ltd. 2008;275.
16. Li W, Dai H, Li Z, Xu AE. Reflectance confocal microscopy for the characterization of mycosis fungoides and correlation with histology: a pilot study. *Skin Res Technol*. 2013;19(3):352-355. https://doi.org/10.1111/srt.12049
17. Mancebo SE, Cordova M, Myskowski PL, Flores ES. Reflectance confocal microscopy features of mycosis fungoides and Sézary syndrome: correlation with histopathologic and T-cell receptor rearrangement studies. *J Cutan Pathol*. 2016;43(6):505-515. https://doi.org/10.1111/cup.12708