REFLECTANCE CONFOCAL MICROSCOPY IN THE DIAGNOSIS OF PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMAS

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Abstract

The article presents a brief review of the literature about the incidence, classification, clinical manifestations and modern methods for the diagnosis and treatment of the Primary Cutaneous Diffuse Large B-cell Lymphomas. The authors present the clinical case of a preliminary diagnosis of the disease with reflectance confocal microscopy before biopsy and following verification of diagnosis with pathomorphological and immunohistochemical studies of the biopsy specimen.

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Introduction

Primary Cutaneous Lymphomas (PLC) are a heterogeneous group of diseases caused by neoplastic cutaneous proliferation of affine to the skin tissue clones of T-lymphocytes, NK-cells, or B-lymphocytes. According to the literature, the incidence of primary cutaneous lymphomas in the world ranges from 0.3 up to 1.18 cases on 100 thousand population and continues to grow. Primary cutaneous B-cell lymphomas account for 10% to 30% of all PLC and are characterized by generally relatively homogeneous clinical course and favorable prognosis (1-3).

The WHO-EORTC (World Health Organization-European Organization for Research and Treatment of Cancer) classification divides the B-cell lymphomas of the skin into five types: primary cutaneous marginal zone B-cell lymphoma; primary cutaneous follicle center lymphoma; primary cutaneous diffuse large B-cell lymphoma, leg type; primary cutaneous diffuse large B-cell lymphoma, other; intravascular large B-cell lymphoma (1, 2, 4, 5).

Primary cutaneous diffuse large B-cell lymphoma, leg type, constitutes approximately 5-10% of all skin B-cell lymphomas. This form of B-cell lymphomas develops predominantly in older women (median age 78 years) as rapidly growing nodules and plaques located on the skin of the lower limbs. The dermis is infiltrated with centroblasts, immunoblasts, and to a lesser extent, centrocytes. Tumor cells diffusely infiltrate the dermis with the replacement of normal tissues and obliteration of...
skin appendages. Infiltration can also penetrate into the subcutaneous fat. The epidermis is usually intact and separated by a zone of unchanged collagen (Grenz zone). Reactive infiltration and stromal reaction are poorly expressed. Tumor cells express CD20+, CD79a+, bcl2+, MUM-1/IRF4+, and FOX-P1+. Five-year survival rate of patients averaged 55% (6-8).

Primary cutaneous diffuse large B-cell lymphoma, other type, coincides in many pathomorphological features with the primary cutaneous diffuse large B-cell lymphoma, leg type, and is characterized clinically by solitary nodules located in other parts of the skin, more often in the head and neck area. Tumor infiltration expresses CD20+ and CD79a+ (4, 7).

The diagnosis process provides for a comprehensive assessment of the clinical picture, an analysis of the results of pathomorphological and immunohistochemical studies of the skin biopsy showing the presence of malignant lymphoid proliferation in the skin. However, performing a skin biopsy is not always feasible due to a number of reasons, including ethical: in the absence of voluntary informed consent of the patient for the manipulation of the tumor, its localization in cosmetically significant areas or in parts of the body that are problematic for the patients.

In this situation, a high-tech method of noninvasive in vivo skin pathomorphological study is of special significance – that is, reflectance confocal microscopy of skin (RCMS).

RCMS is a unique diagnostic method that allows for multiple examinations of different foci in the same patient with a resolution close to traditional light microscopy but without violating the integrity of the skin.

The advantages of this method are:
- Obtaining fast results compared to the classical pathomorphological study;
- The possibility of layer-by-layer 5 μm thick examination of the epidermis and dermis;
- The analysis of the cellular composition of the epidermis and the upper layers of the dermis;
- The study of the vascularization of the papillary dermis, including the diameter of the capillaries;
- The possibility of estimating the tumor infiltration area and the degree of invasion.

In the published literature on the use of the RCMS method in dermatology, data is provided on the description of healthy skin cytoarchitectonics as well as on the pathomorphological changes in the photaging of skin, in skin neoplasms (actinic keratosis, basal cell carcinoma, or melanoma), and inflammatory and infectious diseases (psoriasis, rosacea, onychomycosis, dermatophytosis, and allergic contact dermatitis) (9-13).

Scientific RCMS pathomorphological studies of the epidermis and dermis in patients with primary skin lymphomas are descriptive and scarce in the current world literature, though emphasizing its high diagnostic value and correlation of the results obtained with the data of histological examination of the skin biopsy specimen (14-17).

The Ural Research Institute of Dermatovenereology and Immunopathology conducts a scientific research on the diagnosis capabilities of RCMS method in primary skin lymphomas. As an example of the use of RCMS in the diagnosis of primary cutaneous diffuse large B-cell lymphoma, we describe our own clinical case.

Patient Z.V., female, 67 years old, visited the Institute’s clinic with complaints on a mass on the scalp, painful on palpation.

Anamnesis morbi. She considers herself ill for two years, when she first noticed the appearance of a small element on the skin of the scalp, without any subjective sensations. During the first months, the patient visited a local dermatovenerologist. The correct diagnosis was not detected and the patient was referred to the Ural Research Institute of Dermatovenereology and Immunopathology.

Anamnesis vitale, allergic anamnesis, and professional route are without features. Heredity is not burdened for skin diseases and oncopathology.

Objective exam: A patient of medium height, normal stature, and satisfactory nutrition. Normal breath sounds, no wheezing. Heart sounds are clear, rhythmic, blood pressure is 130/86 mm Hg, HR is 70 bpm. The abdomen is soft, painless on palpation, the liver and spleen are not enlarged. Parotid and submandibular lymph nodes are palpable, oval, and round shaped, of elastic consistency, painless, mobile, up to 1 cm in diameter. Bowel and bladder functions are normal.

Status localis. Unaffected skin areas are of physiological color, normal humidity, and turgor. Visible mucosa is moist, of physiological color. The skin process is represented by a mass of irregular shape, dense consistency, pink-red color, 4*4.5 cm in diameter, localized on the skin of the scalp, in the right occipital area. The focus is infiltrated, rises 0.5 cm above the surface of the skin, has an uneven and nodular surface with partially preserved hair follicles and hair growth (Fig. 1). Hair and nail plates are not changed. Dermographism is red and fast.

Laboratory data. Complete blood count: Hb - 131 g/L, Ery. - 4.21x10¹²/L, Leuk. - 5.5x10⁹/L, Neutr. - 2.8x10¹⁰/L, Eos. - 0.3x10⁹/L, Lymph. - 2.1x10⁹/L, Mon. - 0.3x10⁹/L, ESR - 5 mm/h. Hepatic blood chemistry showed an increase in cholesterol to 6.53 mmol/L.

Data of blood biochemical tests: crude protein - 76.0 g/L, albumin - 42.8 g/L, globulin - 33.2 g/L, bilirubin - 4.6 micromol/L, alanine aminotransferase - 6.6, aspartate aminotransferase - 13.5, glu-
cose - 5.58 mmol/L, cholesterol - 6.53 mmol/L, urea - 3.5 mmol/L, creatinine - 56.3 micromol/L.

Hepatic blood chemistry showed an increase in cholesterol to 6.53 mmol/L.

Urine analysis: colour - yellow, 1010, pH - 6.5, glucose - not detected, protein - not detected, epithelium - not detected, salts - not detected.

Data of immunogram: IgA - 2.66 g/L, IgM - 0.9 g/L, IgG - 10.4 g/L.

Serological *Treponema pallidum* kit is negative.

Antibodies to HIV, hepatitis B and C are not detected.

At the consultation of dermatovenerologists at the Ural Research Institute of Dermatovenerology and Immunopathology, the skin oncopathology was suspected, and an incisional skin biopsy from the focus was recommended. However, given that the patient refused from a skin biopsy and did not sign a voluntary informed consent to this manipulation, we performed RCMS in vivo using a VivaScope® 1500 microscope (Germany).

According to the RCMC data, patient Z.V. had signs of a tumor process: focal axial lengthening of keratinocytes was observed, violating the epidermal and dermal architectonics (the "flow" sign); dilated capillaries with increased blood flow were detected; numerous rounded atypical cells, presumably lymphocytes, were visualized; multiple tumor infiltrates destroying the stromal structure of the dermis were recorded (Figures 2 and 3).

To detect the definitive diagnosis is necessary to realize pathomorphological and immunohistochemical examinations of skin biopsy specimen. The obtained RCMS data allowed us to convince the patient on the necessity of skin biopsy for the
The results of pathomorphological examination of the skin biopsy specimen. Epidermis is without significant changes. In the dermis, a dense diffuse infiltration is noted, without signs of epidermotropism, formed by large lymphoid cells with amphophilic cytoplasm, and rounded and oval nuclei. A high level of mitosis and apoptosis of the proliferating cells is noted (Fig. 4).

Immunohistochemical examination of skin biopsy specimen. Tumor cells diffusely express CD20 (L26) and do not express CD3 (SP7), Bcl-2 (100/D5), CD10 (56C6), or CD30 (Ber-H2). The minimum number of reactive T-cells is noted (CD3+, Bcl-2+, CD20-). The Ki67 index (SP6) is about 90% (Fig. 5). Conclusion: histological structure and immunophenotype of the tumor correspond with the diagnosis of primary cutaneous diffuse large B-cell lymphoma, Bcl-2 negative.

Based on the case history, the clinical and pathomorphological data, and the results of immunohistochemical examination of the skin biopsy specimen, the final nosological diagnosis was established: primary cutaneous diffuse large B-cell lymphoma, the other type, Bcl-2 negative, IA (Ann Arbor). The patient was referred to a hematologist for treatment.

In the city hematological center, two courses of R-CHOP polychemotherapy (Rituximab, Cyclophosphamide, Hydroxydaunorubicin, Oncovin, and Prednisone) were performed, with complete regression of the tumor (Fig. 6), and follow-up by the local hematologist.

Conclusion

The presented clinical case demonstrates that the RCMS can be successfully implemented as a preliminary diagnosis method in the prebiopsy stage since it allows revealing the pathomorphological cytoarchitectonic signs on primary skin lymphomas in the epidermis and dermis without disturbing the integrity of the skin and helps in certain clinical situations argue the necessity of conducting clinical-laboratory examination with histological and immunohistochemical study of the skin biopsy specimen.

Patient informed consent obtained.
Financial disclosure: none declared.
Conflicts of interest: none declared.
Reflectance Confocal Microscopy in the Diagnosis of Primary Cutaneous Diffuse Large B-Cell Lymphomas

Review