Modern diagnostic techniques in dermatology (clinical lecture)

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The article provides an overview of current diagnostic techniques (including morphological and functional methods for assessment of skin structure and condition) used to verify skin and subcutaneous fat diseases.

Keywords: diseases of the skin and subcutaneous fat, microscopy, immunohistochemistry, immunofluorescence, enzyme immunoassay, biomarkers, molecular diagnostics, sequencing and functional testing methods, non-invasive diagnosis.

Clinical diagnosis of the skin and subcutaneous fat diseases within the competence of dermatologists and venereologists is a pressing issue due to, on the one hand, high incidence and, on the other hand, negative effects of these diseases on human health.

In Moscow, the capital city of the Russian Federation, high incidence of skin and subcutaneous fat diseases is registered over the past few years in adult and child population. Thus, skin and subcutaneous fat diseases accounted for 72.5% of the total illness pattern in 2016 (70.3% in 2013, 68.55% in 2014, 73.11% in 2015). The incidence of dermatoses was 3583.7 per 100 thousand population, and the highest incidence rate of this disease was observed in adolescents aged 15—17 years (2016 — 9515.2 cases per 100 thousand population) (Fig. 1).

It should be noted that there are increasingly more diseases that are torpid to conventional therapy and often lead to disability and even death.

By 2016, there has been considerable increase in the incidence of skin tumors (mostly benign, 99.4%), by 431% compared to the data as of 2009 (Fig. 2).

It is known that skin and subcutaneous fat diseases and skin tumors are diagnosed by dermatovenerologists, and visual examination is a primary diagnostic technique, which is based on the observation of primary and secondary morphological elements that ultimately match the clinical presentation of a certain disease. However, in some cases (such as unclear and slight clinical symptoms or similar clinical presentation of some diseases), additional verification is required for the diagnosis.

Diagnostic techniques used to verify the diagnosis of skin and subcutaneous fat diseases can be divided into two large groups: methods for morphological evaluation of skin structure, which in turn are divided into invasive and non-invasive techniques, and functional methods for assessment of skin condition (Fig. 3).

Pathomorphological (histological) study is still the basic laboratory method for verification of the diagnosis of dermatoses and skin tumors. Pathomorphological examination of skin bioplates enables examination of skin structure and detection of changes (patterns) characteristic of a certain dermatosis; it is highly helpful when the differential diagnosis is required. Apart from the conventional light microscopy, phase contrast, interference, luminescence, polarization, stereoscopic, ultraviolet, and infrared microscopy, which are based on various properties of light, are used to study biological objects, including the skin.

In vitro confocal microscopy, an optical laser scanning, which enables obtaining complete high-resolution image of the skin layers in three dimensions (height, width, and depth), is one of the modern pathological method [1].

When talking about the verification of the diagnosis of dermatoses, we cannot ignore electron microscopy, a precise ultrastructural method, which, in turn, may be divided into transmission, scanning, and immuno-electron. In this method, images of studied objects are obtained due to the directed electron flow. However, the analyzed biological material must be prepared in the form of very thin sections because of the low penetrating ability of electrons. However, only electron microscopy can provide direct information about the molecular organization of cells and assess the ultrastructural changes that occur in the skin under the influence of various therapeutic effects [2—5].

Histochemistry, which studies localization of various substances and their metabolites in tissues by dye binding to specific chemical components of cells, is a good supplement to a pathomorphological examination. This method can be used for differential staining of fat, glycogen, nucleic acids, nucleoproteins, enzymes, and other chemical components of cells. Quantitative assessment of the results of reactions is also possible. The use of histochemistry in dermatology can be exemplified by determining the amount of melaniferous cells in the basal and spinous layer of epidermis in vitiligo patients [6]. The presence of glycogen is a common diagnostic criterion of...
hair follicle tumors (clear cell gидroadenoma and eccrine poroma); in this case, all cells of the epithelial sheath and sweat glands contain large quantities of glycogen. Neutral mucopolysaccharides are detected in patients with Paget’s disease [7].

Immunohistochemistry is an immuno-microscopy of tissues, which detects specific substances (antigens). It is currently a popular method for diagnosing skin dermatoses and tumors. The direct method is based on direct specific binding of labeled antibodies to an antigen being detected (Fig. 4).

The indirect method is two-staged (Fig 4). At the first stage, unlabeled primary antibodies bind to a target antigen. At the second stage of the study, the target antigen is detected by interaction between the secondary, labeled, antibodies and primary antibodies, which play a role of antigens for secondary antibodies.

Immunohistochemistry is rapidly becoming a standard procedure in many laboratories and it is available in the form of fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), and silvering in situ hybridization (SISH).

The use of immunohistochemistry in dermatology can be exemplified by its application for diagnosis of mycosis fungoides. Thus, the immunophenotype of tumor lymphoid cells corresponding to mature memory T cells (βF1+ CD3+ CD4+ CD5+ CD7+ CD8− CD45RO+) [8], aberrant phenotype of lymphocytes (decrease in expression of CD2, CD3, CD5, CD7 markers, the loss of pan-T cell antigens) [9], and the number and maturity of
**Immunofluorescence analysis**, an immunological method for detection of surface and intracellular antigens in samples of tissue sections, as well as cell suspensions and blood, is conceptually close to the immunohistochemistry. The method is based on the visualization of antigen using specific antibodies labeled with fluorescent markers. Similarly to immunohistochemistry, immunofluorescence method can be either direct or indirect, and in the latter case it is two-staged. In dermatology, this method is most often used to confirm the diagnosis of pemphigus. In the presence of pemphigus vulgaris, direct immunofluorescence reaction shows fixation of complement (C3) and Ig (G, A, or M) in the intercellular spaces of the epidermis, and the disease is diagnosed based on this fact (Fig. 5) [11,12].

Among the major trends in the development of modern dermatology in the near future, a special role is played by the direction associated with the development and implementation of non-invasive methods for in vivo examination of skin structures [13—20].

These methods include the following. **Dermoscopy** is based on visual assessment of skin lesions for fast verification of the diagnosis (it enables recognition of morphological structures that are invisible to the naked eye).

**In vivo confocal microscopy** using optical scanning laser produces 3D real-time high-resolution images of the skin layers (height, width, and depth).

**Optical coherence tomography** uses an optical near-infrared radiation to probe biological tissue; the method is characterized by high resolution, contrast, and depth of investigation of 1.5 mm.

**Ultrasound examination of the skin** is a highly accurate and reproducible noninvasive method for skin examination, which enables the differentiation of the skin layers, assessment of vascularization state, and operating with “echogenicity” and “echostructure” concepts.

There are a number of methods to assess the functional condition of the skin. Some of these methods enable evaluation of the lipid balance in the skin (sebumetry evaluates the activity of the sebaceous glands), others (mexametry, chromametry) determine the color characteristics of the skin. Skin hydration can be assessed by corneometry and vapometry. **Tevametry** assesses the barrier properties of the stratum corneum, **revisometry** assesses the state of connective tissue fibers of the dermis; **cutometry** and **ballistometry** assess deformation and elastic properties of the skin, measurement of skin pH assesses the status of acid-base equilibrium (Table 1).

Methods for assessing the functional state of the skin are currently widely used in dermatology and cosmetology for examination of patients with acne, rosacea, and other skin diseases and cosmetic defects [21—26].

A so-called **marker diagnosis** is an important direction in the diagnosis of dermatitis. **Biomarker** is an analyte (specific protein, metabolite, gene, RNA), whose characteristics significantly correlate with the physiological, pathological, or clinical manifestations of the disease. The main purpose of targeted “marker” diagnostics is to identify certain molecules or their complexes, which are present only in the diseased tissues or cells and are secreted to the external or internal environment of the body in the case of disease.
Depending on the methodologies used for detection, markers can be divided into “biochemical” and “clinical” (detectable by biochemical and clinical methods), “immunological” (detectable by immunological methods), “molecular or molecular genetic” (detectable by molecular methods), “proteomic” (detectable by proteomic analysis techniques), etc.

Biochemical and clinical methods can be used to detect:

- **rheumatoid factor (IgM autoantibodies interacting with Fc-fragment of IgG),** which are often observed in patients with systemic lupus erythematosus (SLE), having a pronounced articular syndrome [27—28];
- **lupus anticoagulant (LA)** belongs to IgG. LA is a parameter of coagulogram, a group of antibodies to phospholipids comprising cell membranes. On the average, LA is detected in 60% of SLE patients. It was first detected in a SLE patient, which determined its name. The presence of LA in the blood is indicative of the predisposition to thrombosis, whose mechanism is not fully understood [29];
- **LE-cells (lupus erythematodes cells)** are the morphological manifestation of the immunological phenomenon characteristic of SLE. The death of neutrophils is an important characteristic of this phenomenon. Complex autoimmune processes associated with SLE result in cell destruction and formation of structureless spherical entities (hematoxylin bodies) from their nuclear substance (depolymerized DNA), which are then phagocytosed by neutrophilic leukocytes. Thus, LE-cells are the polymor-
phonuclear neutrophils (more rarely, eosinophils or basophils) with phagocytosed cell nucleus or its individual fragments. On the average, these cells are detected in 70 to 80% of SLE patients [30];

“Muscle decay enzymes” (creatine phosphokinase, lactate dehydrogenase, alanine, and aspartate aminotransferase, AST, aldolase) in patients with dermatomyositis [31-33].

Immunological methods are used to detect:

- total IgE in the serum of patients with atopic dermatitis (enzyme immunoassay, ELISA);
- specific IgE/IgG4 antibodies to food, domestic antigens, vegetable, animal, and chemical antigens, detectable in patients with atopic dermatitis (ELISA);
- antibodies to type 1 and 3 desmogleins in patients with pemphigus (ELISA);
- IgG4-antibodies to bullous pemphigoid antigens (230 kD; BPA-1; 60-180 kD; BPA-2) (ELISA) [34—41].

A number of autoimmune diseases, such as SLE, dermatomyositis, scleroderma, and systemic vasculitis are verified by identifying autoantibodies using immunological methods (ELISA, protein microarrays). In this case, anti-double-stranded native DNA antibodies, histones, Sm-antigen cardiolipin, and β2-glycoprotein 1 [42—46] are used as immunological markers. Antibodies against cytoplasmic proteins and ribonucleic acids of the muscle tissue (to aminoacyl-tRNA-synthetases, JO-1-synthetase, Mi-2, Factor I-a, and SRP-antigen) are determined for verification of dermatomyositis [47]. Antibodies to centromere B and topoisomerase I (Scl-70) are the diagnostic indicators of focal and diffuse systemic scleroderma, respectively.

Systemic vasculitis is verified by detecting antibodies to neutrophilic cytoplasmic antigens (ANCA) (cytoplasmic (c-ANCA), perinuclear (p-ANCA)), including antibodies to MPO and serine protease from the a-granules of neutrophils (PR3) are the serological markers of primary systemic vasculitis.

<p>| Table 2. Immunological markers used for diagnosis of autoimmune diseases |
|--------------------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Disease</th>
<th>Marker</th>
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<tr>
<td>SLE</td>
<td>Anti-double-stranded native DNA antibodies, histones, Sm-antigen, cardiolipin, and β2-glycoprotein 1</td>
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<tr>
<td>Dermatomyositis</td>
<td>Auto antibodies to cytoplasmic proteins and ribonucleic acids in muscle tissue (aminoacyl-tRNA-synthetases, JO-1-synthetase, Mi-2, factor I-a, SRP-antigen)</td>
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<tr>
<td>Scleroderma</td>
<td>Antibodies to centromere B and topoisomerase I (Scl-70) are the diagnostic indicators of focal and diffuse systemic scleroderma, respectively</td>
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Dermatoses may be verified using molecular analysis methods, such as polymerase chain reaction (PCR), including a real-time one, hybridization techniques (DNA arrays, qualitative and quantitative analysis: expression microarrays), sequencing techniques from Sanger sequencing to new generation sequencing (NGS). These methods have a number of advantages such as high information content, high speed of the tests, relatively low invasiveness (blood or buccal mucosa imprint are tested), the possibility of simultaneous study of the groups of genes and loci and genome-wide sequencing.

Molecular techniques (PCR) are used to determine clonality of T-lymphocytes (based on the genes of γ- and β-chains of T-cell receptor) and B-cells (based on the genes of the heavy chain of IgH immunoglobulins) for the differential diagnosis of tumor (skin lymphoma) and reactive lymphocyte proliferation (parapsoriasis, pseudolymphoma of the skin, etc.). In this case, detection of monoclonal proliferation is indicative of malignant nature of the disease [59—62].

Timely identification of a comorbid disease, which can significantly burden the course of the underlying disease, is an important approach to the management of patients with dermatoses. For this purpose, high-performance systems for biochemical, hematologic, enzyme immunoassay, ion-selective, atomic absorption analysis, and chemiluminescence are currently widely used based on the large-scale implementation of automation processes. At the same time, there is the trend towards significant increase in the sensitivity of analytical methods due to change in the analyze detection limits (zeptomoles — 1 x 10-21; yoctomoles — 1 x 10-24 per liter), which is indicative of a multifaceted “technological revolution” in laboratory analysts [63]. In the context of the possibility of rapid detection of various pathologies in patients with dermatoses, the high-precision multi-parameter microbead immunoassays (MBIA) should be mentioned. This method is based on the use of flow fluorimetry of polystyrene microspheres labeled with red and infrared fluorophores followed by laser detection and digital signal processing. Analyzed substrates include serum/blood plasma, extracts of skin bioplates, synovial fluid, saliva, biological secrets, and other body fluids. The research
subjects include the markers of diabetes mellitus, acute phase, angiogenesis, apoptosis and sepsis, skin markers (keratins -6, -1, 10, 11, involucrin, fibronectin), immunoglobulin isotypes, markers of cardiovascular diseases; system for determining cellular signals. Moreover, this method can be used to study gene mutations (SNP-genotyping), proteins, nucleic acids, transcription factors. Thus, this analytic method can be used in medical practice and for research.

Personalized, predictive, preventive medicine that studies the possibility of predicting diseases in a particular person on the basis of the revolutionary achievements of the XXI-century biology, which is called a “high-dimensional biology”, is one of the most promising areas of modern molecular medicine, relying on laboratory methods.

The term “personalized medicine” was first used by K.K. Jain [64]. Personalized medicine is an integrative medicine, which includes the development of personalized treatments, testing for predisposition to diseases, prevention, and diagnosis in combination with treatment and monitoring of treatment.

Methodology of predictive personalized medicine is based on the identification of genes, whose certain alleles predispose, or conversely, prevent the development of various diseases, using sequencing (determining the nucleotide sequence) as the basic technique. [65].

The available molecular genetic methods can be used to study the characteristic features of the genotypes of sick and healthy individuals at the level of gene fragments (analysis of mutation and single nucleotide polymorphisms (SNPs); the study of abundance of a gene fragment), the whole gene (full-length sequencing), genome-wide sequencing, and also to study the level of gene expression (transcriptomics).

To date, the structure of genetic risk factors for development of psoriasis (PSORS loci, TNF-A gene, RAGE gene, IL-10 gene; rs1800871, IL12B gene; rs7709212, RUNX3 gene; rs7536201; rs1886734, genes of apoptosis and inflammatory mediators (DR4 Glu228Ala, CaspO Ile479Leu, rs12188300 IL 12B A/T, and TRAF3IP2 Trp74Arg); psoriatic arthritis (IL23R gene; rs7530511; rs11209026; IL12B gene; rs3212227; rs6887695; G (Arg) allele of DR4 Lys441Arg gene, and T allele of rs12188300 polymorphism of IL 12B A/T gene), atopic dermatitis (mutations in filaggrin gene, rs1800795 of IL6 gene, DEFBI gene), and SLE (TNFSF4, CD44, IRF8, MIR-146A, TMEM39A genes) was partially solved owing to the so-called Genome-Wide Association Studies, as well as more local research using sequencing techniques [66—76].

Currently, the genetic basis of many genodermatoses, in particular, epidermolysis bullosa, Bloch-Sulzberger syndrome, and a number of others are known due to the use of molecular methods and sequencing techniques, which enable verification of the diagnosis in disputable cases (Table 3).

Molecular markers can be used to personalize the early diagnosis (for example, identification of histocompatibility antigens HLA-B8 and HLA-DR3, linked with the production of myositis-specific antibodies in patients with dermatomyositis), prediction of metastasis of malignant tumors of the skin, the so-called preventive genomics and micro RNA-omics [83—85].

It is well known that therapy of dermatosis can result in pronounced therapeutic response, have no clinical effect, or cause complications. Pharmacogenomics addresses the issues of prediction of the efficacy and safety of treatment and studies gene polymorphism, which determines the variability of the individual response of the human body to the pharmacotherapy. For example, Russian and international publications describe the predictive value of determining TNR-R-II gene polymorphism, expression level of some chemokines, their receptors, and CCR9 protein, and the presence of HLA-Cw6 antigen in psoriasis patients in terms of efficacy of therapy with genetically engineered drugs (infliximab, ustekinumab, etc.) [86—88].

Therefore, the current repertoire of the Russian and international dermatology includes both conventional and innovative, invasive and non-invasive analytic methods, which enable clinicians to verify the diagnosis in questionable cases, carry out early diagnosis, predict the course of the disease and pharmacological response to therapy. Automation of routine procedures, miniaturization of the study format, and combining various modules into integrated multifunction systems lead to rapid increase in productivity of biological studies in dermatology and their rise to a new level.

There is no conflict of interest


