ABSTRACT

Cytogenetic testing is one of the most important diagnostic criteria for many leukemias and lymphomas. Specific genetic changes detected by karyotyping and fluorescence in situ hybridization may be predictive of the course of the disease and enable effective treatment. In the present era of targeted therapy, personalized patient treatment is becoming increasingly important. The rising incidence of cancer as well as the progress in diagnosis and effective therapy generates the need to carry out cytogenetic examinations in an increasing number of patients. The Polish base of cytogenetic hematooncology laboratories is generally sufficient, but could function more effectively. The condition for improvement is the introduction of effective quality control procedures and a coherent legal system, including clear rules for referral and financing of such tests. Close cooperation between cytogenetic diagnosticians and hematologists is essential for effective treatment of hematological patients.

Keywords: cytogenetics, karyotype, FISH, diagnosis, treatment, leukemia, lymphoma

Słowa kluczowe: cytogenetyka, kariotyp, FISH, diagnostyka, leczenie, białaczki, chłoniaki

1. CYTOGENETICS AS A SCIENCE SUPPORTING THE TREATMENT PROCESS

Testing methodology

It is universally accepted that changes in genetic material have direct consequences for the functioning of the cell. Advances in knowledge and testing techniques have led to increased usefulness of cytogenetic assays, as they now enable the detection of a considerable number of genetic defects, both hereditary (clinical cytogenetics) and formed de novo in the neoplastic process (oncological cytogenetics).

Highly specialized cytogenetic hematooncology laboratories perform assays that make it possible to detect and evaluate genetic abnormalities specific to the neoplastic tissue cells collected from the patient. Cytogenetic tests detect such changes in genetic material and reveal a connection between characteristic aberrations and the processes of cancer formation and development. During cell division (in metaphase), DNA is organized in chromosomes, while for the rest of the time it remains in the form of loose nuclear chromatin (interphase nuclei). Chromosomes can be examined by karyotyping. A morphological point of reference is the normal chromosome pattern (An International System for Human Cytogenetic Nomenclature, ISCN 2009). Certain changes in chromosome morphology, such as translocations or deletions, are indicators of specific genetic defects. Molecular cytogenetic methods (fluorescence in situ hybridization, FISH) enable the evaluation of uncoiled DNA strands in interphase nuclei and the detection of defects in particular genes. Their interpretation, based on current knowledge and diagnostic principles, reveals possible clinical consequences.
of such defects and is of fundamental importance in diagnosing leukemias and lymphomas, and also often in making a prognosis and planning therapy for these diseases.

**Karyotype analysis**

Cytogenetic analysis involves fragments of tissues with neoplastic changes. In karyotyping, it is necessary to culture neoplastic cells *in vitro* to obtain the division phase. Then, the analyst may evaluate the morphology of stained (“banded”) metaphase chromosomes and determine any deviations from their normal number and structure. Appropriately prepared material is analyzed under a light microscope. Karyotyping is not an automated process. The popular image analysis systems equipped with a “karyotyping module” offer only preliminary evaluation. In detailed analysis, the final decisions are made by the diagnostician, whose knowledge and experience are critical to correct interpretation of the obtained chromosomal band pattern. Standardized results describing all observed changes are given in accordance with nomenclature standards and publications of scientific societies. It should be remembered that while karyotype analysis allows for evaluation of the entire genome of a leukemic cell, it reveals only those aberrations in which the size of rearranged material is at least several million base pairs.

Thanks to band analysis, it is possible to conclusively determine the presence or absence of diagnostically significant translocations, such as t(9;22)(q34;q11), t(8;21)(q22;q22) or t(8;14) (q24;q32), in the blood or bone marrow cells of a leukemic patient. Karyotype analysis makes it also possible to detect genetic factors that affect the course of the disease, such as deletions of chromosomes or their fragments (e.g., 7/del(7q)) in myeloid leukemias.

**Fluorescence in situ hybridization (FISH) using specific DNA probes**

Fluorescence in situ hybridization (FISH) enables the detection of defects in genetic material not only during metaphase, but also in the interphase nuclei of the studied cells. This technique makes it possible to identify relatively small changes, corresponding to the size of the gene analyzed. A reference DNA segment stained with a fluorophore is called a probe. The probe binds specifically to the complementary DNA sequence of the patient’s neoplastic cell, and the dye it carries allows for the detection of the studied DNA fragment under a fluorescence microscope. The use of several probes at the same time shows the location and mutual relationships between the studied genes. An additional advantage of this method is that it can be employed for examining archival material (paraffin sections). A FISH assay concerns only regions complementary to the DNA probe used. Such an assay is qualified by many factors (the margin of error depends, among others, on the type of probe used and kind of cancer; the number of cells analyzed should be related to the type of defect; etc.). In typical cases, diagnosis can be made relatively easy, but a full interpretation of results requires considerable experience.

The FISH technique is used to conclusively determine the status of the examined genes (fusion, rearrangement) in neoplastic cells. The presence or absence of certain gene fusions, e.g., *BCR/ABL, PML/RARA, RUNX1/RUNXI*, or rearrangements within genes, e.g., *MYC* or *CCND1* is a diagnostic indication. FISH analysis also makes it possible to evaluate the number of copies of genes (deletion, amplification), which is important in assigning patients to high risk groups, e.g., for multiple myeloma (*TP53* deletion or *FGFR3/IGH* fusion).


In hematological diagnostics, the methods described above are often used in parallel, because diagnosis based on only one of them may be insufficient or incomplete. A precise evaluation of the status of selected genes in the cell does not convey it possible to formulate a reliable opinion. Only the mutually complementary results of karyotyping and FISH analysis make it possible to formulate a reliable opinion.

2. **Clinical usefulness of cytogenetic assays in hematology**

**Diagnosis and prognosis**

Access to cytogenetic testing, awareness of its potential, and good cooperation between the clinician and the cytogeneticist in diagnosing leukemias and lymphomas cannot be overestimated. In leading hematological centers around the world, cytogenetic assessment is a standard element of the diagnostic process. The current classification of malignancies of the hematopoietic and lymphatic systems, published in September 2008 by the World Health Organization (WHO), is mostly based on genetic abnormalities, and distinguishes leukemias and lymphomas with given morphological, cytochemical, immunophenotypic, and clinical features. An understanding of the genetic and molecular aspects of cancers led to reclassification of many leukemias and lymphomas as compared to previous categories.

The main field of exploitation of cytogenetic assays is the diagnostics of leukemias. The first practical application of cytogenetics to cancer concerned chronic myeloid leukemia (CML). The identification of the specific translocation t(9;22) (q43;q11) and subsequent research proved that the fusion gene BCR/ABL, present in the abnormal Philadelphia chromosome (Ph) is the cause of CML rather than merely its marker. Thus, the presence of t(9;22) in the marrow cells of a patient with suspected CML is of fundamental diagnostic importance. Most acute myeloid leukemias (AML) also reveal the presence of specific recurrent genetic abnormalities, such as t(15;17), t(8;21), inv(16), and inv(3). These diagnostic chromosomal aberrations and the corresponding genetic defects, which are critical to diagnosis, can be detected in cytogenetic laboratories. Similarly, in the case of lymphoid neoplasms, cytogenetic aberrations such as t(8;14), t(11;14), and t(14;18) are used to confirm diagnosis in the most widespread group of these diseases which affect B cells.

The genetic features of a cancer are determined at two levels:

1. in cytogenetic laboratories, which detect chromosomal aberrations by karyotyping and genetic abnormalities by FISH;
2. in molecular diagnostic laboratories, which investigate changes in individual genes or their products (mutations, quantitative and qualitative transcript determination).

A typical molecular diagnosis is of great significance in the selection of therapy, mostly in solid tumors, enabling the detection of, e.g., abnormalities of HER2, KIT, or KRAS genes. Selective molecular tests related to the treatment of hematopoietic malignancies involve, e.g., B- and T-cell clonality in the diagnostics of some lymphomas, or the presence of mutations in JAK2, PDGFR, or FGFR1 genes in myeloproliferative neoplasms.

In certain diseases, the presence of genetic abnormalities (e.g., the presence/duplication of the Ph chromosome or the presence of del(17p)) is of significant predictive value. The detection of such abnormalities is a recognized method of predicting treatment outcomes: for instance, the presence of the Ph chromosome in a precursor lymphoid malignancy indicates a poor prognosis. The diagnostic standards involving cytogenetic assays developed by international research groups are reflected in recommendations of expert groups in particular countries. Cytogenetic assays can

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determine chromosomal aberrations in neoplastic cells and identify those whose presence leads to certain clinical outcomes: the development of a leukemia or lymphoma, the course of the disease, and even its sensitivity or resistance to a particular kind of treatment.

**Treatment and treatment monitoring**

The end of the 20th century saw significant progress in the treatment of hematopoietic neoplasms. Thanks to the introduction of combined therapies, marrow transplants, and biological therapies, the length and quality of the patients’ lives has been greatly improved. An example of a medicine used in “biological therapy” is all-trans-retinoic acid (ATRA), which brings very good results in the treatment of acute promyelocytic leukemia with t(15;17) chromosomal translocation and PML/RARA gene fusion.\(^{10}\)

A breakthrough in the approach to treatment of leukemias was the introduction of an entirely new type of therapy – molecularly targeted therapy. The first effective medicine of this kind is Imatinib (a tyrosine kinase inhibitor, also known under the trade names of Gleevek and Glivec). This medicine is now used routinely in the treatment of chronic myeloid leukemia. As an inhibitor of the abnormal protein BCR/ABL1, Imatinib attacks only those cells that contain the product of the abnormal BCR/ABL1 gene.\(^{11}\) The use of Imatinib with chemotherapy is also beneficial in the treatment of children with acute lymphoblastic leukemia with BCR/ABL fusion.\(^{12}\)

Imatinib and other kinase inhibitors may be administered only if the patient’s bone marrow cells contain the molecular target of treatment, that is, the oncogenic protein. Thus, from the point of view of the clinician, of fundamental importance is to be absolutely confident that the patient will be responsive to the medicine prior to treatment implementation. And this kind of information is provided by cytogenetics, using two routine techniques: FISH and karyotyping.

CML therapy with tyrosine kinase inhibitors is a long-term treatment aimed at elimination of cells with the BCR/ABL fusion (Ph+). Under the circumstances, of particular importance is regular monitoring of the course of treatment. The frequency and scope of cytogenetic monitoring of CML is precisely determined. Apart from the time of diagnosis, it should take place every 6 months following the implementation of therapy, and annually following complete cytogenetic remission (CCyR).\(^{13}\) After completely eliminating Ph+ (that is, attaining CCyR), molecular monitoring should be implemented using RT-PCR or RQ-PCR to assess further reduction of the number of neoplastic cells. Such regular surveillance makes it possible to assess changes in the proportion of Ph+ cells in bone marrow and/or detect a recurrence of cells with BCR/ABL fusion in patients in remission. Cytogenetic testing (karyotype/FISH) may confirm the efficacy of the therapy, or indicate the time at which it becomes ineffective. The appearance of Ph+ cells in patients with cytogenetic remission reveals the onset of secondary resistance and means that the medicine administered should be replaced with other TK inhibitors, such as Nilotinib or Dasatinib.

In the case of lymphomas, the most frequent type of treatment remains chemotherapy. The critical issue is to choose the right kind of therapy: more intensive in patients with highly aggressive neoplasms, and less intensive in those patients for whom tests show the absence of negative predictors with a high degree of certainty. It is now possible to successfully treat a large proportion of patients with non-Hodgkin lymphoma, which is more malignant and has a more aggressive course. However, some groups of patients (e.g., with Burkitt lymphoma) show resistance to conventional treatment since the very beginning, but good results may be achieved with high-dose

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chemotherapy. In such cases, the application of an aggressive course of treatment will be successful only if such a protocol is implemented immediately. Also here, a diagnosis of BL is confirmed by the cytogenetically detectable rearrangement of the MYC gene or t(8;14)(q24;q32) translocation.14

In the case of indolent lymphomas, which are less malignant, it is extremely difficult to achieve a complete recovery. The physician’s role is often limited to regular observation, with therapy implemented if the disease is found to progress. Progression most often occurs in patients with predictors of poor prognosis, such as TP53 gene deletion. Aberrations are reflected in the karyotype and/or FISH image and may be routinely determined by hematological cytogenetic laboratories.

The future of oncology is individual targeted therapy, that is, medicines matched to the needs of particular patients based on certain features of their neoplastic cells. There are still relatively few medicines that can be called truly “targeted.” Currently, they primarily include tyrosine kinase inhibitors and, to some extent, monoclonal antibodies.

Monoclonal antibodies (e.g., rituximab, alemtuzumab, and bortezomib) use the mechanism of blocking certain receptors and are administered mainly in the treatment of various types of lymphomas. Another medicine in this category is the monoclonal antibody trastuzumab (Herceptin), which is effective against breast cancer involving an abnormal HER2 gene.

It is well known that targeted therapy may be effective only in those patients whose neoplastic cells exhibit the molecular feature that is the target of the therapy. Such abnormal features can be detected by cytogenetic tests, which allow for definitive selection of the target group of patients. Confirmation of the presence of the molecular target (defect) in the patient’s neoplastic cell should be a prerequisite for implementing targeted therapy, as the decision to prescribe a given antibody depends on the genetic subtype of the diagnosed disease.

A full cytogenetic examination provides extensive information concerning changes within the entire genome of the neoplastic cell, including the presence of key aberrations. However, one must not forget that such an assay is not equivalent to a high-resolution molecular test performed using PCR techniques (e.g., BCR/ABL transcript level in a CML remission). The diagnostic ideal would be then to have full access to tests conducted at both molecular and cytogenetic laboratories, which should cooperate with hematological clinics. In Poland, limited access to highly specialized laboratories of this kind leads to a situation where some patients are being treated too intensively, while others may receive an insufficient or inadequate treatment, including expensive molecularly targeted therapy.

Cost of treatment

Modern anticancer therapies are generally expensive. Molecularly targeted therapies cost between PLN 10 000 and 20 000 per patient per month. The continuous progress of science gives rise to hope that new, efficient medicines targeted at some molecular defects in neoplastic cells will be developed, but as a result the cost of the therapy will increase. The spectacular effectiveness of the modern treatment methods (in some groups of patients) leads to pressure to use them on a more widespread scale. Thus, in all health care systems, two contradictory trends emerge: greater use of targeted therapies in oncology versus reduction of expenditures (limited reimbursement of state-of-the-art oncological pharmaceuticals).

In the near future, one can expect an increasing number of dilemmas concerning the application of potentially effective therapies in individual patients. From the ethical and economical points of view, the best solution is an objective selection of a group of patients in whom a given kind of therapy is very likely to be successful. One could even argue that if the above condition is met, targeted therapy may be cheaper than the treatment of potential complications or progression of the disease. However, to meet this condition, targeted therapy must be preceded by genetic diagnostics.

Genetic testing in cancer cases is included in the list of medical procedures reimbursed by the National Health Fund. The item Comprehensive genetic diagnostics of neoplastic diseases specifies the methods that may be used for examination of genetic material,15 these include: classical

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14 Zarządzenie Nr 68/2009/DSOZ Prezesa Narodowego Funduszu Zdrowia z dnia 3 listopada 2009 r. w sprawie okreslenia warunków zawierania i realizacji umów w rodzaju świadczenia zdrowotne kontraktowane odrębnie 96.1 KB, 3.11.2009 r. Załącznik nr 3: Warunki udzielania świadczeń w rodzaju: świadczenia zdrowotne kon-

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cytogenetics and molecular cytogenetics (FISH), as well as other tests selected on the basis of indications. The price of such a diagnostic procedure is currently underestimated. However, even if one takes into account the actual cost of cytogenetic testing, it is relatively low as compared to the cost of pharmaceuticals. Systematic implementation of the obligation to cytogenetically detect the genetic defect in neoplastic cells prior to introducing targeted therapy may enable savings in the system of financing hematopoietic therapy. An example for the effectiveness of such a solution is the current system of reimbursement of tyrosine kinase inhibitors (Imatinib and other TKIs), which requires that in chronic myeloid leukemia a cytogenetic assay be performed prior to the implementation of treatment (diagnostics) and during therapy (monitoring).

More extensive implementation of such diagnostic procedures may limit the total cost of pharmaceuticals purchased, while increasing the individual effectiveness of therapy by selecting patients sensitive to a given type of treatment. A relatively cheap cytogenetic or molecular assay can conclusively determine whether the proposed treatment is likely to succeed, that is, whether the medicine will encounter its molecular target in the neoplastic cells. Regular monitoring of the patient throughout the treatment process will signal the right moment for discontinuation or change of therapy. Thus, greater use of cytogenetic and molecular testing will enable the rationalization of treatment costs and improve the ratio of medical spending to health benefits (lengthening of the patient’s life or a complete recovery).

3. DEMAND FOR CYTOGENETIC TESTS IN HEMATOONCOLOGY

Poland has a population of over 38 million. In 2008, over 131 000 cases of cancer were recorded among adults, but the National Cancer Register suggests that these data are an underrepresentation of the actual figure (an estimated 156 000 + cases per year). According to predictions for the following two decades, in 2025 the number of cancer cases may increase to as many as 170,000. Leukemias and lymphomas account for 5-10% of all malignancies, so some of these patients will require genetic testing, including specialized cytogenetic diagnostics. The various types of myelo- and lymphoproliferative neoplasms affect persons of all ages; they range from childhood leukemias to old-age lymphomas.

Chronic myeloid leukemia most often occurs in 40-50-year-olds, but it can also affect children, young adults, and elderly persons. The current data concerning the CML incidence and treatment in Poland are not exact. The number of new cases is estimated at 350 per year. Prior to the introduction of Imatinib, CML patients survived 3-5 years following diagnosis. Due to the fact that now the survival time has been significantly extended, the number of living patients with this disease has grown. The International Randomized Study of Interferon and STI571 (IRIS) showed that after 7 years of monitoring the survival rate was 94% (excluding deaths from non-hematological causes). The introduction of second-generation tyrosine kinase inhibitors has made it possible to continue treatment in those patients in whom Imatinib is no longer effective. According to the guidelines of the National Health Fund, a prerequisite for the implementation and continuation of treatment with Imatinib (and other TKIs) in CML patients is a cytogenetic assay for the presence of the Philadelphia chromosome or the BCR/ABL1 fusion. Furthermore, therapy should be monitored every half a year or every year. Even today, over 1000 cytogenetic tests are estimated to be required by CML patients alone. Given the minimum survival time of 10 years and over 300 new diagnoses a year, this figure will soon become many times greater.

Chronic lymphoid leukemia affects mostly...
patients aged 55-60, while the risk of developing it increases after the age of 40. The most frequent type of this disease is B-cell CLL (over 90% of cases), which is also the most widespread form of leukemia in Europe. The presence of some cytogenetic factors (predictors of poor prognosis) in neoplastic cells indicates an aggressive course of the disease. Cytogenetic evaluation is currently required for clinical examination, and is also recommended at the time of diagnosis in all new cases. The cytogenetic stratification of patients into the various risk groups entails certain clinical consequences, e.g., the possibility of early detection of disease progression and implementation of effective treatment. The annual incidence rate amounts to 3.5/100,000 and increases with the age of the patient. Thus, it is to be expected that in the coming years the annual incidence of CLL will reach over 1,500 cases potentially requiring cytogenetic evaluation.

Acute leukemias constitute approximately 40% of all leukemias in adults. The Polish Register of Incidence of Acute Leukemias in Adults recorded 2109 new cases in the years 2004-2006. The diagnosis and treatment of acute leukemias should be conducted in specialized facilities following detailed determination of all risk factors. This group of patients will require cytogenetic and molecular diagnostics as the basis for diagnosis. According to the 2008 WHO guidelines, such tests are necessary for assigning patients to the various risk groups, selecting the optimum management strategy, and also monitoring the course of therapy.

Acute myeloid leukemia consists of a number of subtypes, for which the incidence rate in Poland amounts to 2.5/100,000 persons aged 18 and over, while in children this illness is diagnosed in 50-60 patients annually. The total number of patients requiring cytogenetic evaluation as part of AML diagnostics may be in excess of 1000 persons a year. As management strategies are becoming more and more effective, the number of patients to be monitored is expected to grow many times over.

The incidence of acute lymphoblastic leukemia (ALL) is 2/100,000 persons. In children, it is the most prevalent type of leukemia, affecting about 250 of the youngest patients a year in Poland. According to the latest WHO diagnostic guidelines from 2008, in diagnosis of ALL cytogenetic and biomolecular assays must be performed (along with cytomorphological and immunophenotypic tests). A health care facility treating such leukemias should have access to prognosis-relevant tests, that is, karyotype testing for the presence of chromosomal aberrations such as t(9;22) or t(4;11). Similar recommendations are included in the protocols of the Polish Adult Leukemia Group (PALG). The results of karyotyping are the basis for selecting a course of therapy involving tyrosine kinase inhibitors. The above data show that demand for cytogenetic testing may here amount to about 1000 new cases of ALL a year.

Lymphomas account for about 4% to 5% of all malignancies. The annual incidence of cancers of the lymphatic system is over 3000, out of which the majority are NHLs. Over the past decades, the incidence of lymphomas has been on the increase. In 2008, more than 2600 new NHL cases were recorded. In this group of diseases with varied etiology, morphology, and course, the current classification takes into account the results of new clinical, immunological, molecular, and genetic studies. Recent years have also seen considerable

advances in the treatment of lymphomas, including progress in targeted therapy. Therefore, demand for genetic diagnostics is going to rise, especially in respect of B-cell lymphomas.

In summary, every year there are over 5000 new cases of leukemias and over 2600 new cases of lymphomas in Poland. Given the above figures, the demand for cytogenetic diagnostic tests in cases of suspected myelo- and lymphoproliferative diseases is 8000 to 10,000 annually. Furthermore, every year an increasing number of patients will be given effective therapy requiring cytogenetic monitoring of outcomes. As a result, another 10,000+ patients may need cytogenetic diagnostics each year.

4. CYTOGENETIC LABORATORIES IN POLAND

Requirements

Just like all diagnostic laboratories, also cytogenetic hematooncological laboratories must meet the requirements imposed by law. The Laboratory Diagnostics Act and regulations of the Minister of Health specify requirements for the premises and equipment of a laboratory, as well as professional requirements for the personnel and management. These regulations also define the conditions of performing laboratory procedures, imposing on laboratories the obligation to formulate and adhere to procedures for ordering tests and for collection, transport, and reception of specimens. Laboratories should also implement appropriate testing methods as well as ensure an adequate quality of laboratory procedures. Detailed standards for the quality of work are given in Annex 3 Quality standards for medical laboratory genetic procedures, evaluation of their quality and diagnostic value, and interpretation and authorization of test results by the laboratory.

Quality assurance requires laboratories to control the quality of tests, present them in an adequate manner, and maintain appropriate documentation. Furthermore, the special nature of genetic assays and responsibility for the results reported mean that laboratories must observe the guidelines of European cytogenetic associations and continually update the knowledge and methods applied to keep abreast of advances in the field of hematooncology.

The current state

According to data from the Hematooncological Cytogenetics Section of the Polish Society of Human Genetics, whose membership consists of cytogenetic laboratories specialized in hematooncological diagnostics, currently there are about 20 such laboratories in Poland. These include large laboratories employing 10 to 20 workers, as well as small ones (some of them with only 1 worker). All of these laboratories employ some certified laboratory diagnosticians (from 1 to 13) and have been issued a record number by the National Chamber of Laboratory Diagnosticians. In most cases, laboratory managers are specialized in the field of medical or clinical laboratory genetics. Only a few laboratories are not able to ensure continuous availability of a highly qualified diagnostician (those having fewer than 2 certified diagnosticians). Half of the laboratories have been doing hematooncological tests for at least 10 years, while only a few were established recently (1 to 5 years ago). According to regulations, laboratories are required to perform at least 200 tests of a given type per year in order to maintain proficiency, but not all of them meet this criterion.

29 Rozporządzenie Ministra Zdrowia z dnia 3.03.2004 r. w sprawie wymagań, jakim powinno odpowiadać medyczne laboratorium diagnostyczne. Dz.U. 43.408; Rozporządzenie Ministra Zdrowia z dn. 23.03.2006 w sprawie standardów jakości dla medycznych laboratoriów diagnostycznych i mikrobiologicznych. Dz.U. 06.61.435; Rozporządzenie Ministra Zdrowia z dnia 21 stycznia 2009 r. zmieniające rozporządzenie w sprawie standardów jakości dla medycznych laboratoriów diagnostycznych i mikrobiologicznych (Dz.U. 09.22.128).


and quality control of diagnostic procedures, the Hematooncological Cytogenetics Section of the Polish Society of Human Genetics launched a project “Organization of Reference Diagnostic Cytogenetic Hematooncology Laboratories.” The project is aimed at improvement and standardization of the quality of diagnostic procedures in member laboratories.

The Section holds annually The National Workshop of Hematooncological Cytogenetics, which is a forum for exchange of experience and education of cytogeneticists working in the field of hematooncology. The workshop involves both lectures and practical exercises as well as presentation of the results of tests conducted by all participating laboratories. The Standardization Committee of the Section issued a publication containing basic guidelines for laboratory work.

All workshops are focused on improving quality standards (in accordance with recommendations of professional organizations and relevant legal regulations and principles).

Under the Organization of Reference Diagnostic Laboratories Project, competence tests (inter-laboratory testing of control samples) for the FISH technique have been conducted annually, beginning in 2007. The method of control samples is the basic technique for evaluation of precision and accuracy in laboratory diagnostics. A group of laboratories perform a test on the same sample and compare their results. The competence test is aimed at improving the quality of FISH assays, enhancing their clinical usefulness, education of the diagnosticians, and verification of the reliability of the results. Participation in such a competence test makes it possible to evaluate a laboratory’s ability to perform a given type of test. Test results are discussed in detail at a “Working Meeting,” during which the participating laboratories are given an assessment of their results and a certificate of participation.

Generally speaking, the quality of genetic tests is found to be constantly improving. The Section is planning to implement a similar competence test for evaluation of karyotyping expertise. This measure is aimed at organizing a network of cytogenetic hematooncological laboratories recommended by the Polish Society of Human Genetics.

Problems and difficulties

Hematooncological cytogenetics evolved as a separate domain of cytogenetics, and it has been developing very dynamically over the past 10 years. Unfortunately, the current regulations in the Polish health care system are not always consistent with the methods of cytogenetic testing in the field of hematooncology. Standards as to the quality of work in cytogenetic laboratories are defined in Annex No. 3 to the Regulation of the Minister of Health of January 2009. These guidelines are binding for all cytogenetic laboratories, both those examining constitutive karyotypes and cancer cell karyotypes. However, analysis of the annex clearly shows that the law does not recognize the different nature of malignancy diagnostics. Laboratories are obligated to collect family history in terms of genetic burden and provide consultations with a clinical geneticist. While this is indeed necessary for clinical genetic laboratories, it does not have any practical value in hematooncological diagnostics, which examines changes immediately linked to the neoplastic process, that is, acquired ones. The obligation to prepare and implement principles for determining the source of chromosomal aberrations found to enable appropriate assessment of risk in the family is inadequate for the same reasons.

Cytogenetic testing for the needs of hematooncology should be reimbursed by the National Health Fund (NFZ), which specified to this end Separately Contracted Service no. 5.10.00.0000041 – Comprehensive genetic diagnostics of neoplastic diseases. However, the requirements defined by the NFZ are a compilation of requirements for constitutive karyotype testing and molecular testing (mostly for genetic predispositions), which is not fully consistent with

examination of neoplastic material. As a result, the NFZ requirements concerning cytogenetic hematooncological diagnostics are confusing. For example, annex 4 to the Regulation 68/2009/DSOZ of the President of the NFZ concerning the conditions of signing and executing contracts such as health care services contracted separately demands the inclusion in the panel of diagnostic methods also molecular biology, which is necessary for diagnosing hereditary diseases. Thus, all the laboratories offering such services are required to be able to routinely provide predispositional testing, even though the overwhelming proportion of hematooncological neoplasms are not inherited.

The valuation of cytogenetic hematooncological services proposed by the NFZ is not adequate to the costs actually borne. The NFZ description of the services allows for the use of a variety of techniques, including: classical cytogenetics, high resolution cytogenetics, molecular cytogenetics (FISH), molecular biology (PCR and its modified versions, RFLP, SSCP, etc.), and other tests selected depending on indications. Such a comprehensive procedure is valued at about PLN 500, while the cost of a full cytogenetic examination amounts to at least PLN 1000. The use of several DNA probes in FISH (e.g., the basic panel for the stratification of myeloma involves 4 probes) also increases the cost of the procedure. Moreover, a separate molecular test represents an additional cost of a few hundred to a thousand PLN (depending on the method). However, taking into consideration the benefits of genetic testing, its costs are still relatively low, and the popularization of this method may bring considerable savings in the treatment of leukemias and lymphomas.

Health care providers may fail to order genetic diagnostics due to problems with access to such tests as well as due to financial considerations. It is difficult to obtain information about the location and competencies of cytogenetic diagnostic laboratories, and about the services they offer. There are widespread administrative difficulties in the area of settling accounts between laboratories and their external clients (physicians). Furthermore, determination of the genetic profile of a neoplasm does not always imply approval for the implementation of a given course of therapy. It should also be noted that not all physicians are convinced as to the immediate usefulness of cytogenetic assays. In the basic medical education system, there is scant information about the potential of cytogenetic and molecular diagnostics. Therefore, most physicians are not fully aware of the importance of such data. Obviously, during their specialization training, specialist physicians acquire considerable knowledge of genetic diagnostic methods in a relevant field; however, this knowledge is not always put into everyday practice.

**Summary**

Cytogenetic testing has gained a permanent place in hematooncological practice. The contribution of cytogenetic assays to the diagnostic process, increases the likelihood of achieving improved treatment results or a full recovery in patients with a determined (also genetically) malignancy. However, in the Polish health care system, the availability of reliable genetic testing for hematooncology is far from perfect. The current financing of diagnostic procedures in the field of cancer genetics is quite insufficient. Many health care providers limit the scope of tests ordered from highly specialized laboratories for want of funds. On the other hand, at a time of strong competition in the medical services market and a lack of control mechanisms, there are concerns as to the standards of some of the tests offered. Under the current tendering system, in which the only criterion of awarding contracts is price, low prices may be offered at the cost of quality. Another problem, which is equally important, is the fact that some physicians are not convinced as to the need to perform specialized cytogenetic examinations.

Under the circumstances, some remedial measures ought to be taken in the main areas specified below:
1) developing a general set of indications for genetic testing as part of modern diagnostics of leukemias and lymphomas – such guidelines should also enable the implementation of the latest treatment options in defined groups of patients;
2) reform of financing – the diagnostic procedures and point values defined by the National Health Fund (NFZ) do not reflect the actual amount of work performed by the laboratories or the costs borne by the health care providers that order the tests;
3) creating a base of specialized laboratories and improving diagnostic standards by developing a system of competent laboratories as well as their registering and certifying;
4) disseminating knowledge of modern treatment options and diagnosis strategies as part of basic medical education and during specialist oncological training.

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