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Use of genetic analysis for prognostic stratification of differentiated thyroid cancer

Relevance. *The share of thyroid cancer among malignant neoplasms is rapidly growing worldwide. Although the mortality from thyroid cancer is not high, the patients still face the risk of recurrence. Therefore, it is very important to identify the patients prone to relapse and to adapt their treatment accordingly. This is the main goal of risk stratification. However, no method of risk stratification has proven its superiority. Some genetic mutations are associated with thyroid cancer, and their role in risk stratification is now under study. The consideration of mutation status can improve the stratification of mortality and risk of relapse for thyroid cancer.*

Purpose. *This paper justifies the objective of a local study which aims to identify prognostic markers of differentiated thyroid cancer (DTC) in order to improve the patient stratification system. The planned retrospective case control study will include the analysis of archival materials, medical records, electronic databases of patients, and the new generation sequencing of biopsy materials of 200 patients with DTC treated in 2012-2015.*

Results. *The purpose of the planned study is to create a single risk stratification system for patients with DTC on the basis of identified mutations associated with the aggressive forms of DTC.*

Keywords: *differentiated thyroid cancer (DTC), risk stratification system, genetic mutation.*

The share of thyroid cancer among malignant neoplasms is rapidly growing worldwide. Though mortality from thyroid cancer is low, the risk of recurrence in most cases reaches 30% [1]. Therefore, it is very import- ant to identify the patients prone to relapse and to adapt their treatment plan accordingly. The risk of relapse is stratified after surgery by determining the stage of thyroid cancer using different systems, i.e., those developed by the American Joint Committee on Cancer (AJCC) or Union for International Cancer Control (UICC). The AJCC/UICC staging system is designed to be used to predict disease-specific mortality; however, it is limited in its ability to account for variables that can affect long-term prognosis [1]. Patients who are considered to be at high risk are burdened with frequent monitoring which might be unnecessary if they do not relapse. And vice versa, patients who were originally in a low-risk group may not respond to treatment. Several other risk stratification systems such as EORTC, AGES, AMES, MACES, and MSK include other forecast predictors [1]. However, none of these methods has proven its superiority.

Recently, the role of incorporating genetic mutations and molecular markers into risk stratification systems is under study [1-9]. Some genetic mutations are known to be associated with thyroid cancer. Progress has been made in studying the genetic mechanisms of thyroid cancer. Molecular tests are expected to predict the risk of cancer better than cytological tests. The discovery of new gene mutations is also expected to affect clinical management decisions and the choice of therapy (surgery or drugs). Optimization of treatment as new data become available requires constant update of risk assessment. Dynamic risk stratification systems will allow predicting clinical outcomes better than permanent risk assessments.

BRAF and other mutations and molecular markers were found to be associated with the clinical course, relapse and / or prognosis of thyroid cancer; and the significance of these findings is now studied and evaluated. With the new knowledge about the gene features of thyroid cancer, their role in risk stratification is growing.

Therefore, it is still unknown which cases require more aggressive treatment, the use of radioiodine therapy, or simply a safe resection of the lobe of the gland. This study aims to determine effective markers for thyroid cancer risk stratification. The results of such study could be applied both nationally and internationally. The goal of this study is to identify prognostic markers of differentiated thyroid cancer (DTC) with the aim of improving the patient stratification system.

Methods. The first stage of the planned retrospective case control study will include the analysis of archival materials, medical records, and electronic databases of patients with verified papillary and follicular thyroid cancer. The second stage will include the selection of biopsy materials of 200 patients treated in 2012-2015 and the new generation sequencing of samples using the Illumina TruSight cancer set. The currently available technology of nucleotide sequencing allows analysing a lot of genes and single polymorphisms at once providing even more accurate diagnostic data. This amount shall be sufficient to calculate the reliable difference between the groups.

Inclusion criteria: thyroid cancer primary diagnosed in 2012-2015; papillary carcinoma; follicular cancer; surgery on the thyroid; age – 18 to 70 years; availability of status data in the Electronic Registry of Cancer Patients of the Republic of Kazakhstan; informed consent of the patients for the use of their biological materials.

Exclusion criteria: undifferentiated cancer; medullary cancer; age below 18 years or above 70 years; symptomatic treatment only; refusal to participate prior to the start of the survey.

All possible measures will be taken to protect personal data of patients. Data that can represent or dis- close personal data will be encrypted or deleted during publishing. The study is non-invasive and safe for the patients as it involves only the archival materials of patients from the

medical records in paper and electronic form. It is better that the patient is aware of a possible prognosis of his disease for the possibility of adjusting the dispensary treatment. The study will follow the ethical principles of the Helsinki Declaration of Patient Safety [10], and the GCP and GLP principles for studies involving people in accordance with the legislation of the Republic of Kazakhstan.

Discussion. One of the most common mutations in thyroid cancer is in the BRAF gene; it leads to activation of the MAPK pathway. The BRAF mutation is observed in about 45% of patients with papillary thyroid cancer (PTC) [11] related to DTC. Meta-analysis of the results of 14 studies (a total of more than 4,000 patients) showed that the BRAF mutation in patients with PTC was associated with 2.66 times higher mortality [1, 12]. Another study showed that the relapse of PTC at an average of 36 months occurred in 20.9% of patients with the positive BRAF mutation vs. 11.6% of patients with the negative BRAF mutation [1, 13]. The meta-analysis conducted in 2012 showed the credible association of BRAF mutation in PTC with a higher risk of recurrence and spread of the tumour [1, 14].

The second most common mutation is the RAS gene mutation. The RAS gene has three isoforms: HRAS, KRAS, and NRAS. Although RAS is a well-recognized dual activator of MAPK and PI3K pathways, in tumour genesis RAS mutations – are much strong activators of the PI3K-AKT pathway. The RAS mutation is associated with 2.9 times' higher mortality from thyroid [1, 12]. The RAS mutation was detected in 30-45% of patients with follicular thyroid cancer (type of DTC) and in 30-45% of patients with follicular variant of PTC [11].

Molecular changes in the RET / PTC gene also play an important role in the carcinogenesis of the thyroid gland. They are often found in patients with PTC and Hurtle cell thyroid cancer. The frequency of such changes was also found to increase the possibility of developing thyroid tumours after irradiation [11].

In the studies, the BRAF, RAS (especially, NRAS), ALK, PIK3CA, PIK3CB, PDPK1, AKT1, AKT2, and TERT gene mutations were associated with a more aggressive course of the disease (spread beyond the thyroid, more advanced stages of thyroid cancer, short survival, distant metastases, and a weaker response to therapy) [11]. In addition, new physical changes in oncogenes (MDM2, FLI1), the transcription factors and repressors (MITF, FLI1, and ZNF331), the epigenetic enzymes (KMT2A, NSD1, NCOA1, and NCOA2) and the protein kinases (JAK3, CHEK2, and ALK) were identified [9]. In a very recent study of GWAS, single polymorphisms in the NRG gene were identified.

In addition, the researchers have confirmed three previously reported loci (FOXE1, NKX2-1 and DIRC3) and have identified seven new loci of susceptibility (VAV3, PCNXL2, INSR, MRSB3, FHIT, SEPT11 and SLC24A6) associated with the thyroid gland [8].

As we can see, the molecular changes and the gene mutations play an important role in the aggressiveness of thyroid cancer. In recent studies, new previously unknown mutations of genes were identified whose prognostic significance is yet unknown. The idea of this study is to create a single risk stratification system for patients with DTC on the basis of identified markers (mutations) associated with the aggressive forms of DTC.

Conclusion. The expected identification of informative markers will allow developing a more in-depth and efficient system of disease progression risk assessment and the stratification of patients by risk groups based on genetic analysis. This will promote the choice of patient-oriented treatment and the limited use of aggressive methods of radionuclide therapy only for the needy patients.

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