BRIEF SYNTHESIS OF THE COUNTRY REPORT IN ROMANIA

Report created in the framework of the project Promoters of advanced oncogenetics open online training and multimedia raise awareness on multidisciplinary assessment of patients and their families at risk of hereditary or familial cancer, Reference number 2018-1-RO01-KA202-049189, Strategic Partnerships for vocational education and training Erasmus+ programme

Romania faces a challenging epidemiological situation that request to initiate measures for the monitoring and implementation of prevention strategies in oncological pathology. Over the last decade, there is an increased attention focused on hereditary cancer problems. There are many research projects at national or international level addressing this issue, as well as an increasing number of Healthcare Centers that provide molecular testing and services for patients where the involvement of hereditary factors is predicted.

1. The current epidemiological situation on cancers, in general, and on hereditary cancers (breast, ovarian, colorectal cancer and other) in Romania

1.1. Incidence

According to the International Agency for Research on Cancer (IARC), in Romania, in 2018, the estimated total number of new cases of cancer for both sexes at all ages was 4,313,123. The distribution of the cases by sites indicates that breast cancer was the most common (12.3%), followed by colorectal cancer (11.8%), lung cancer (11.2%), prostate cancer (10.6%), bladder cancer (4.7%), skin melanoma (3.4%), renal cancer (3.2%) and other cancers (42.8%). Among the other cancers that were recorded we mention: uterine cancer (2.9%) and ovarian cancer (1.6%) (GLOBOCAN 2018). There is an upward trend in the number of new cases for all types of cancer.

1.2. Prevalence

The 2008 Annual Report of the North-East Regional Registry of Cancer shows a prevalence of cancers in Iasi county of 15.08 per 100,000 inhabitants, which is below the average of the North-East Region (16 / 100,000 inhabitants) and a specific cancer mortality of 207.73 per 100,000 inhabitants, well above the average of the North-East Region (188.38 / 100,000

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inhabitants). In 2018, IARC published for Romania an estimated number of prevalent cases (5 years) of cancer for both sexes at all ages of 12,334,517 (GLOBOCAN 2018). Case distribution by location indicates that breast cancer was the most common (16.9%), followed by prostate cancer (12.7%), colorectal cancer (11.6%), bladder cancer (5.2%), lung cancer (4.1%), skin melanoma (4%), uterine cancer (3.7%) and other cancers (41.6%). Among the other cancers that were recorded we mention: ovarian cancer with 1.6%.

1.3 Risk factors

In the North-East region of Romania, the following factors were associated with an increased risk of developing ovarian cancer: age (OR = 12.44; p <0.01x10-5) (58.63% of cases were diagnosed at ages between 50 and 69 years, which means during postmenopausal period); origin in urban area (OR = 3.45; p = 0.04x10-3); ovulatory period over 30 years (OR = 10.84; p <0.01x10-5) and age at diagnosis (OR = 2.01; p=0,016x10^{-3}). In contrast to most research founded in the literature, the authors did not identify the early age at first menstruation, late menopause, obesity, smoking, and high socioeconomic status as factors that increase the risk for ovarian malignancies, although the results of the current study were in agreement with some conclusions published by other authors.

1.4. Mortality

According to the International Agency for Research on Cancer (IARC), in Romania, in 2018, the estimated total number of cancer deaths for both sexes at all ages was 1,994,380 Deaths distribution by location indicates that lung cancer was on the first place (20%), followed by colorectal cancer (12.5%), breast cancer (7.1%), pancreatic cancer (6.6%), prostate cancer (5.5%), gastric cancer (5.3%), liver cancer (4%) and other cancers (39.1%) (Fig. 5). Among the other cancers that were recorded we mention: ovarian cancer (2.3%) and uterine cancer (1.5%) (GLOBOCAN 2018). A rate of 8.23 deaths per 100,000 women (which far exceeds the country average) was recorded for North-East Region.

2. The current situation on the National Strategies about the models used in genetic risk assessment for hereditary cancers

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According to Cancer Screening in the European Union Report on the implementation of the Council Recommendation on cancer screening no programme had been initiated in the following three member states: Bulgaria, Romania and Slovak Republic.

There are significant differences between Romania and Western European countries regarding the detection, treatment and survival rate in cancer diseases. Of all risk factors for cancer, hereditary predisposition is the only risk factor that reaches positive or negative predictive value, justifying a medical and oncogenetic monitoring. Oncogenetic monitoring of HBOC patients and the recommendation for a BRCA molecular genetic testing are made based on general inclusion criteria, such as: 3 or more cancer patients in the same family line, regardless of gender. Exceptions to this rule may be accepted if there are cancer cases diagnosed at early onset (before the age of 40), multiple cancers, and medullary or triple-negative breast cancers – for its high association with germline BRCA mutations. HNPCC patients monitoring is mainly based on the reunion of 3 criteria defined in Amsterdam in 1991 and reviewed in 1999: (1) at least three affected family members diagnosed and with reduced Lynch syndrome histologically proved (gastric, small bowel, ovarian, urothelial, pancreatic, or bile duct) cancers; (2) at least 2 first degree affected relatives in 2 different generations; (3) at least one of 5 affected individuals receiving such a diagnosis at age <50 years.

Before 2010, there was no information on BRCA mutations, sequence variants, or polymorphisms in the population of Romania. The first characterization of this population started with a cumulative effort of Gr. T. Popa University of Medicine and Pharmacy in Iasi, Alexandru Ioan Cuza University of Iasi, Auvergne University and the Jean Perrin Centre in Clermont-Ferrand (France). Molecular analysis performed for a small number of predisposing families was possible both because of the technological partnership with the French team, especially because of the local development of rapid methods of mutational pre-screening.

There is no National Oncogenetic Healthcare System in Romania. And consequently there is no National Strategies for Diagnosis and oncogenetic management of hereditary cancer patients. Diagnosis of hereditary risk for breast / ovarian cancer through BRCA genes has been performed on couple of hundreds at risk patients and only in a context of scientific research, although the genetic counselling is a vital need for monitoring cases with this pathology. The screening of hereditary gene predisposition genes is currently a standardized practice in Western

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European countries, a practice that allows tailor-made monitoring and genetic counseling for those at risk in these families.

There are also no National approved risk assessment models available in Romania. There was a prospective study conducted at IOCN Cluj that evaluated the performance of the Myriad risk assessment model for 250 high-risk breast cancer pts tested for BRCA1/2 mutations between February 2015 and December 2016 at IOCN. The Myriad genetic risk model can be an acceptable risk assessment tool for determining the risk of carrying BRCA mutations in Romanian population if the score is between 10-20%. The inaccuracy in carrier prediction using Myriad model represents a challenge worthy of additional investigation and comparison with other genetic models. Genetic counsellors should recognize this limitation when using Myriad model and recommending genetic testing for Romanian high-risk breast cancer patients.

3. The current practical situation on Genetic Testing Availability for HBOC and CCR in Romania

According to "Cancer Screening in the European Union Report on the implementation of the Council Recommendation on cancer screening" in Romania the access to genetic testing is provided by a pharmaceutical company. The evaluation of current situation on cancer genetic testing and counselling availability was made by "ROHEALTH - Clusterul pentru sanatate" (the health cluster) association. They conducted a survey regarding the institutions that provide genetic testing using their own network in order to identify the potential responders. The analysis of the answers received from 19 providers who agreed to complete the questionnaire revealed a very limited availability of cancer genetic testing and genetic risk evaluation for hereditary cancer in Romania. Moreover, the Healthcare entities interested to provide genetic testing services for population have little or no information about genetic diagnosis of cancer and molecular evaluation of hereditary cancer risk. Only 2 responders: Genetic Center (private entity) and Pius Brinzeu Emergency County Clinical Hospital (governmental entity) both from Timisoara reported availability of hereditary cancer genetic testing for diagnosis. Other few healthcare entities like Synevo Laboratories, Bioclinica, Medlife, Regina Maria and only one governmental institution - Cluj Oncological Institute provide genetic testing for breast/ovarian and colorectal cancer.

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4. The current situation on Research programs on mutation screening and founder mutation for HBOC and CRC.

Research programs on mutation screening in HBOC and CRC patients

4.1. ROMCAN . Genetic epidemiology of Cancer in Romania Project financed through the SEE Mechanism. The ROMCAN Project ("Genetic Epidemiology of Cancer in Romania") proposes a systematic evaluation of genetic risk factors associated with breast cancer in female (BrCa), colon and rectum (CRC), prostate (PrCa) and lung (LuCa) cancers, representing almost half of the overall burden of cancer in the country, in the Romanian population, aiming to define high risk groups for whom specific preventive measures can be implemented. We also aim to examine if there is any effect modification of the genetic risk by ethnicity, focusing on the Roma ethnic group.

4.2. European Union FP7 Program (ProMark project 202059). Genetic prostate cancer variants as biomarkers of disease progression. The first major objective of PROMARK was to test if inherited genetic variants can serve as biomarkers for prostate cancer prognosis or treatment selection. The second major objective was to perform genomic and functional analysis of predictive variants in order to shed light on the patho-physiology of disease progression.

4.3.Clinical trial information: NCT02317120. ESR-14-10102/17.10.2014 ASTRA ZENECA Ctr.ESR-14-10102/17.10.201. BRCA1 and BRCA2 Mutation in Romanian Population: a Study of Genotype - Phenotype Correlation at Diagnosis With Prospective Disease Outcome and Survival. The aim of the project is to determine types and frequencies of BRCA 1 (B1) or BRCA2 (B2) mutations in high-risk Romanian breast cancer patients, as there is no data published in this population. This prospective study evaluates the germline *BRCA1/BRCA2* mutations in 200 Romanian high-risk breast cancer patients tested between February 2015-January 2017 at IOCN.

4.4. Optimization and implementation of molecular biology technology in depistation of breast and ovarian cancer hereditary predisposition. Project PN-II-ID-PCE-2008, code 1990/2008. The BRCA1 and BRCA2 genes are responsible for a large percentage of hereditary breast and ovarian cancer (HBOC) families. It was proposed the implementation at Iasi of

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modern methodology in molecular biology, in order to develop molecular diagnosis for BRCA mutations in HBOC families.

4.5. Comparative study of gene expression, in healthy and tumoral tissues, in carriers of mutations and polymorphisms of predisposition genes to breast and ovarian cancer. Project CNCSIS-RU-PD 557/2009. Although there are particular characteristics of phenotypes associated with BRCA neoplasms, little information is available concerning the direct consequences of different BRCA mutations and polymorphisms on the expression in the tumor of other cell cycle regulating genes as p53, p21, ATM, CHEK2, Rad50, Rad51. Also, little is known on correlations between regulation pathways involving these proteins, while gene expression represents the perfect instrument in understanding such correlations.

4.6. Development of a multitechnological approach to evaluate the hereditary risk to colorectal cancer (MULTITECHLYNCH). Project CNCSIS-RU-PD 557/2009. The study proposed the development and implementation of a combined multitechnological approach in order to rapidly and accurately evaluate the hereditary risk to colorectal cancer. Our model will combine germinal and tumoral (somatic) analysis, by molecular mutational screening of MMR and BRAF genes, as well as microsatellite instability, promoter hypermethylation analysis and immunohistochemical investigations on MMR proteins. The benefit of the model will either concern patients, families and public health system.

4.7. CHRONEX-RD *:* Involvement of oncogenetics activities within the project "The East European Network of Excellence for Research and Development in Chronic Diseases CHRONEX-RD"

4.8. International research applications not granted

- *Inherited prediSposiTiOn and genetic suscePtibility to Breast Cancer in ethnically diverse EUropean populations (STOP BC in EU). ERA-NET o*n Translational Cancer Research (TRANSCAN) Joint Transnational Call for Proposals 2012 (JTC 2012) on: "Translational research on primary and secondary prevention of cancer"

Project Director - Sheeba University, Israel; Position: Partner - Romania (UMF Iaşi).

- Development of Oncogenetics in Romania (RONCOGEN). PN-II-ID-PCE JOINT PROPOSALS France-Romania 2012 (Bilateral Projects Romania-France tip Brâncuşi). Position: Partner - Romania (UMF Iaşi).

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- Whole Genome sequencing in Greek and Romanian patients with hereditary breast cancer negative for mutations in BRCA1 & BRCA2 genes (SEQGENGRERO). PN-II-ID-PCE JOINT PROPOSALS Greece-Romania 2010 (Bilateral Projects Romania-Greece). Position: Partner - Romania (UMF Iaşi).
- Development of a set of tools enabling the interoperability of different systems (EHR, EDC, LIMS) from different centers. Demonstration on two medical cases : neurodegenerative diseases and inherited cancers (MedDecisionPIPE). Call FP7-ICT-2009-4 Small or medium-scale focused research project (STREP). Project Director: Soluscience, France. Position: Partner Romania (UMF Iaşi).

5. List of institutions, cancer centres etc. that provide genetic testing and counselling services in Romania

ROHEALTH survey also revealed a lack of information on availability of cancer genetic counselling centres. It could means a real lack of this kind of services provided in Romania at this moment. Only 2 of this kind of centres was mentioned by ROHEALTH survey responders; One is Oncogen – Centre for Gene and Cellular Therapies in the Treatment of Cancer is the first state-of-the-art research centre for gene therapies in Romania, dedicated to research in areas that are closely connected to clinical branches of the Pius Brinzeu Emergency County Clinical Hospital Timisoara. The second is Department of Oncogenetics at “Grigore T. Popa” University of Medicine and Pharmacy – Iasi.

All responders appreciated as useful the possibility of developing a network of specialized units to identify individuals at risk for hereditary cancer. These units would provide both molecular testing and further clinical management of identified cases.

The usefulness of these networks would result from a specialized surveillance of families with hereditary cancer risk and would significantly influence personalized therapy in existing cases. They considered being important to include the activities of the oncogenetic centers in a National Program financed by the Public Health Ministry so that the addressability, accessibility monitoring of the patients and their families could be achieved.

6. The situation of Education Programs regarding Oncogenetics:

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ROHEALTH evaluation also revealed a lack of training programs in Oncogenetics for medical staff and for the general population. Only one respondent mentioned that such programs were carried out at the initiative of a private medical unit (Genetic Center). Another postgraduate course for teaching stuff organized by University of Medicine and Pharmacy Carol Davila, Bucharest were mentioned. There is no much available information on research programs correlated with the educational ones in the field of Oncogenetics. The respondents mentioning the cross-border project Romania-Serbia (project coordinator - the Oncogenic Center) and several projects funded by the EU.

The educational programs conducted by Department of Oncogenetics at "Grigore T. Popa" University of Medicine and Pharmacy – Iasi.

For students. Scientific Oncogenetic club for students and Presentations in International Congress for Medical Students and Young Doctors (Congressis 2014 - Iași Romania, 10-13 April 2014 and Congressis 2015 - Iași România, 1-5 April 2015). **For doctors.** Oncogenetic postgraduate course organised by UMF Iași and Advanced School of Ontogenetics (Slănic-Moldova, Romania, 10-14 April 2013 and Gura Humorului, Romania, 30 October -1 November 2014. **For general population.** Webpage of the Oncogenetics Department (www.oncogenetica.umfiasi.ro) ; Information for doctors and patients: Oncogenetic information brochure for doctors; Oncogenetic information leaflet for patients

7. The identification of the practical needs (infrastructure, human and financial resources) in order to establish and to develop an Oncogenetic Network

The Molecular Diagnostic Laboratory is organized in 3 main areas:

- ***7.1. DNA extraction area (pre-PCR area)***
- ***7.2. PCR amplification and DNA sequencing area (PCR area)***
- **7.3.** ***Capillary electrophoresis / interpretation area (post-PCR area)***

Conclusions

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The cancer morbidity and mortality rates are significantly higher in Romania compared to other countries in the European Union. The threat that several more frequent hereditary cancers (colorectal cancer, breast cancer and ovarian cancer) represent to, both male and female population health is too serious to ignore. The efforts of recent years was focused on hereditary cancer risk assessment in order to address early genetic cancer diagnostic and prevention given the evolving roles of citizens, addressability of the patients and health professionals increased interest for new molecular methods for diagnosis. Principles of oncogenetic was promoted especially in Oncological Centers and Universities trough research dissemination at congresses and conferences, professional training programs, cornerstones of all medical progress hat must drive improvements in service delivery across these areas. Thus, the creation of national oncogenetical center network integrated into a similar European structure to fill a void in cooperation, collaboration and shared experiences among countries with similar needs in order to align national health policy with EU- strategies are needed more urgently now than ever.

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