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METHODS FOR *EGFR* VARIANTS ANALYSIS IN NSCLC PATIENTS

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ABSTRACT:

Lung cancer (LC) is the leading cause of death in the world. NSCLC (non-small cell lung cancer) is the predominant form of LC. In the last decade, research and treatment of NSCLC are improved based on molecular analyses used for the determination of the EGFR (epidermal growth factor receptor) variants. To update the findings from the search we performed ten years ago using just the PubMed database, we recently used 3 electronic databases: Web of Science, PubMed, and Scopus. The aim was to reveal the most often used methods to detect EGFR variants for NSCLC diagnostics, as well as to see the application of new techniques in the last ten years. In the first search from 2000-2011 within 292 papers were identified as eligible, and among various methods, PCR methods were identified in 45.2%. Results from a recent search showed that among 987 selected articles published from 2010-2020 showed that PCR methods were identified in 73.05%. Usage of methods for clinical diagnosis depends on many factors, prevalently economic circumstances. The methods of choice for EGFR variant identification are PCR combined with direct DNA sequencing.

Keywords: *PCR, EGFR, NSCLC, lung cancer*

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1. INTRODUCTION

Non-small cell lung cancer (NSCLC) is a prevailing form of the lung cancer, that is still the deadliest cancers in the world [1]. The epidermal growth factor receptor (EGFR) is the key molecule in lung cancerogenesis. The EGFR protein is ususally overexpressed, *EGFR* gene amplified, polymorphic and affected with somatic mutations many epithelial cancers [2-4]. The most examined *EGFR* variants with diagnostic and prognostic potential are mutations in 18-21 exons of the *EGFR* gene, and single nucleotide polymorphisms (SNPs): -216G>T (rs712829), CA repeat (rs11568315), rs712830 (-191C>A), and 181946

C>T, D994D (rs2293347) [5-7]. This paper aimed to review the implementation of standard and novel methods for the detection of *EGFR* variants in clinical research from 2000-2020.

2. LITERATURE SEARCH AND RESULTS

Previously, a literature search was performed from 2000-2011 involving Pub Med database with focus on EGFR mutations [8]. In a subsequent study [9], the findings were updated by searching for the studies published from 2010 to 2020 in the three electronic databases: ISI Web of Science, Pub Med, and Scopus. Details of the search methodology and identification of eligible studies, with precise inclusion and exclusion criteria, have already been published [8, 9]. Both studies present the application of the methods over the years, but here we present their percentage usage counted to the total number of eligible studies 292 [8] and 987 [9] (not in the total number of the identified methods).

Within 292 papers identified as eligible, PCR methods were found in 45.21% (Figure 1). Other methods identified were i.e. immunohistochemical analysis (IHC) in 30.48%, denaturing high performance liquid chromatography (dHPLC) with 4.45%, direct DNA sequencing (DS, refering to standard Sanger sequencing) with 14.73%, and recently developed (RD) 5.14% (Figure 1).

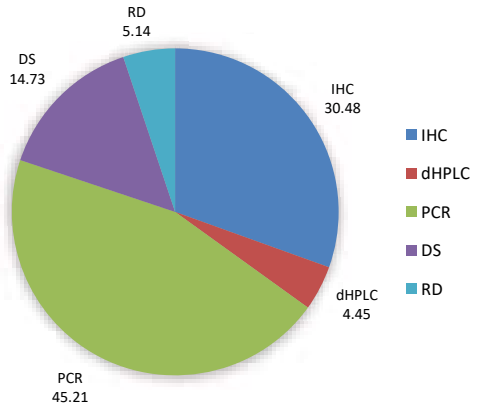

Fig.1. Percent* of methods for EGFR variants detection in the literature search from 2000-2011

Legend: *counted in the 292 total number of eligible articles; dHPLC-denaturing high performance liquid chromatography, DS-direct DNA sequencing, IHC-Immunohistochemical analysis, RD-recently developed.

The following study expanded the search not only for EGFR mutations, but also for *EGFR* single nucleotide polymorphisms that have proven clinical significance [5-7, 9]. A final 987 articles were analyzed and among them PCR methods were identified in 73.05% (Figure 2). Other methods identified for *EGFR* variant detection were 12.06% IHC, 7.50% hybridization methods, 40.53% direct DNA sequencing, 19.35% next generation sequencing, 4.96% proteomics, 3.44% recently developed methods (Figure 2).

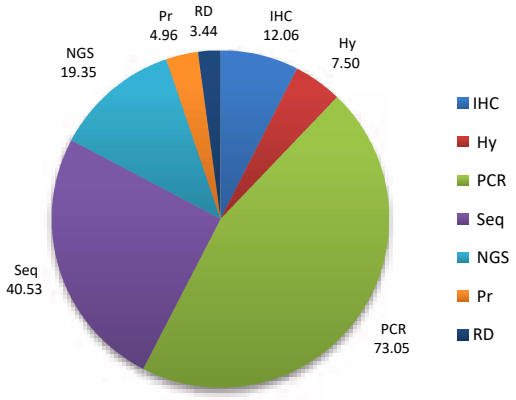

Fig.2. Percent* of methods for EGFR variants detection in the literature search from 2010-2020

Legend: *counted in the 987 total number of eligible articles; IHC-Immunohistochemical analysis, Hy-hybridisation methods, Seq-direct DNA sequencing, NGS-next generation sequencing, Pr-proteomics, RD-recently developed methods.

2. DISCUSSION

In two previous reviews, the methods used in clinical research for identifying the *EGFR* variants in NSCLC patients have been analyzed and compared for the past two decades (2000-2011) and (2010-2020). It was presented here the simplified percentual methods usage in general, not in terms of year of application, or region in the world, as published before [8, 9]. In the two previous studies, it was elaborated on the advantages and disadvantages of screening and target methods applied in twenty years of research [8, 9]. In clinical use, conventional methods were cost effective, with fast results, and much more convenient for diagnostics of target *EGFR* mutations-deletion of exon 19 and point mutation of exon 21 [9-11]. As a result, in 2000-2011 30.48% of the studies applied IHC (Figure 1), but in 2010-2020 they were applied as follows: 12.06% IHC, 7.50% hybridisation methods, and 4.96% proteomics (Figure 2).

The detection of *EGFR* variants relies still on standard techniques, direct DNA sequencing and PCR. In the period of 2000-2011, PCR methods were found in 45.2% and 14.73% direct DNA sequencing of total 292 identified papers (Figure 1). As of 2010-2020, PCR and sequencing dominated clinical research applications with 73.05% and 40.53%, respectively (Figure 2). Their cost-effectiveness and adaptability made them widely applied to the detection of *EGFR* variants [8, 9, 13]. It was evident that the adaptability of PCR methods was of great importance during the Covid-19 pandemic [15].

As fundamental and clinical research progresses throughout the world, advanced methods have emerged. NSCLC treatment in advanced stages often requires approaches like surgical resections or biopsies. After those invasive procedures, DNA from tissue samples is analysed to identify EGFR somatic mutations, affecting 18 to 21 *EGFR* gene exons. Parcitcularly the intracellular tyrosine kinase domain is altered in the EGFR receptor and promoting signals toward cancerogenesis [5-7, 16, 17]. Therapy involving the tyrosine kinase inhibitors (TKIs) were effective in preventing the spread of the aberrant signals in carriers of these somatic mutations. Unfortunately, mutations connected with resistance appeared shortly after TKI implementation [16-18]. Therapy efficacy may also be affected by other *EGFR* variants, such as germline SNPs that can alter *EGFR* gene regulation. *EGFR* polymorphisms were significantly associated with survival, progression-free survival (PFS), and overall survival (OS) in NSCLC patients treated with EGFR-TKIs [19, 20]. On the other hand, different genetic susceptibility to lung cancer was evident considering *EGFR* variants among different ethnic populations [21]. In the future, it will be challenging to identify *EGFR* molecular germline markers with easy and reproducible methods that could replace the diagnostic or prognostic potential of EGFR mutations. Recent liquid biopsies could detect EGFR mutations from blood which contains circulating free tumor DNA (ctDNA) [22, 23]. But beside blood, samples like saliva, sputum, urine, pleural secretions, could analyse ctDNA by using quantitative polymerase chain reaction (qPCR), digital PCR (dPCR) or digital droplet PCR (ddPCR), or next generation sequencing (NGS) [22-26]. *So instead of complex re-biopsy of NSCLC patients, EGFR* resistance mutation could be identified *with this less invasive liquid* biopsies techniques which is important for accurate therapy desicions. For example, if the common EGFR T790M resistance mutation is confirmed, osimertinib is prescribed, othervise chemotherapy i recommended [24]. Advanced methods have high sensitivity and specificity, and capacity to detect novel variants of clinical importance in a small sample size [8, 9, 12, 22, 23]. Besides EGFR mutations, the other genetic alterations panel was examined by NGS methods: KRAS, NRAS, BRAF, PIK3CA, MET and ERBB2 mutations relative to EGFR-TKI resistance [25]. That novel methods were evidenced here

in smaller percent of 3.44% (Figure 2). It is likely that these constraints in the application of those advanced methods stem from economic factors as well as limitations on equipment and/or expertise for low- and middle-income counties [8, 9, 13, 14, 24].

3. CONCLUSION

The PCR and sequencing methods were mostly used in clinics for the detection of *EGFR* variants in NSCLC patients. Many advanced methods have emerged, but their costs often limit their broad use.

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