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Review

Clinical impact of ki-67 labeling index in non-small cell lung cancer

Jan Nyrop Jakobsen*, Jens Benn Sørensen

Department of Oncology, Finsencentre, Rigshospitalet, 9 Blegdamsvej, 2100 Copenhagen, Denmark

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ABSTRACT

The ki-67 index is a marker of proliferation in malignant tumors. Studies from the period 2000 to 2012 on the prognostic and predictive value of ki-67 labeling index (LI) in non-small cell cancer (NSCLC) are reviewed. Twenty-eight studies reported on the prognostic value of ki-67 index with various endpoints. No consensus on the prognostic value of ki-67 LI was found among the published studies neither according to disease stage nor histological subtype. Comparison of studies is hampered by differences in patient populations, methodologies and cut-off values. Five studies explored the predictive value of ki-67 to chemotherapy and none revealed significant influence. Ki-67 index seems to be of prognostic influence in NSCLC although largely variable cut-off levels have been used in the various studies and standardization of methodology is required. The relative importance of ki-67 compared to newer biomarkers has not been explored. It is likely that a signature of several biomarkers in combination may be necessary to more sufficiently stratify patients to various treatment options than is currently possible, especially when it comes to the question of the optimal use of classical chemotherapy. A predictive impact of ki-67 to treatment in NSCLC remains unclear.

1. Introduction

Lung cancer accounts for 20–25% of all cancer deaths and is the most common cause of cancer death [1]. The choice of treatment modality e.g. surgery, radiation, or systemic treatment, is based on prognostic factors among which TNM stage is the most important [2]. Despite treatment according to the prognostic TNM staging system, some patients experience disease recurrence following curative intended treatment and for patients treated for advanced disease there are wide variations in treatment outcome. Thus there is a need for additional prognostic factors to further refine prognostication in order to choose between various treatment options in certain subgroups.

Standard treatment in advanced NSCLC is platinum-based doublet chemotherapy which however may be limited by common toxicities [3]. Response rates to first line chemotherapy in advanced NSCLC range from 15% to 50% [4] and the median survival time from 7.4 to 10.3 months with 1-year survivals around 30% [5,6]. In order to improve treatment, the choice of targeted treatment is increasingly based on predictive factors such as histological subtype or biomarkers, e.g. epidermal growth factor receptor mutations [7] or EML4-ALK gene rearrangements [8]. No biomarkers predicting sensitivity to chemotherapy have so far been implemented in clinical practice.

1.1. Ki-67 labeling index (LI)

Several molecular biomarkers have been investigated for a prognostic or predictive role in NSCLC. One of these is the ki-67 LI, which despite discovered 30 years ago and massively investigated in several malignant diseases remains inconsistent in its indications and interpretation as a clinical marker. The ki-67 protein was originally defined by a monoclonal antibody ki-67 created through the immunization of mice with nuclei of the Hodgkin lymphoma cell line L428 [9]. The name ki-67 is derived from Kiel which is the city of origin and the number of the original clone in the 96-well plate. The biological function of the ki-67 protein, which is encoded by a gene located on the long arm of chromosome 10 (10q25), has not been well determined. But it seems that ki-67 is associated with ribosomal RNA transcription [10] and thus, inactivation of ki-67 leads to inhibition of ribosomal RNA synthesis [11]. Ki-67 is widely considered a marker of proliferation, which is mainly expressed during the active phases of the cell cycle, i.e. G1, S, and G2 and mitosis [12]. However, minor amounts of ki-67 can also be detected in quiescent cells [10].

High proliferation rate is a hallmark of cancer and high ki-67 LI and thus proliferation is reported to predict poor survival in multiple myeloma [13], prostate cancer [14,15], and breast cancer [16,17]. Tumors with high ki-67 are more aggressive and invasive

* Corresponding author. Tel.: +45 35453545; fax: +45 35456966.
E-mail address: jan.nyrop.jakobsen@rh.regionh.dk (J.N. Jakobsen).

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in glioma [18], bladder cancer [19], and anal cancer [20]. Although several studies suggest a prognostic value of ki-67 they often use different cut-points and incomparable patient populations and thus ki-67 often fails to be an independent prognostic factor in multivariate analyses [16,17,21].

The European Neuroendocrine Tumor Society (ENETS) and WHO has recommended the use ki-67 LI of 20% to differentiate between moderate and low differentiated gastroentero-pancreatic neuroendocrine tumors (GEP-NET) [22], which impacts on treatment decision. The majority of studies indicate a prognostic role of ki-67 in GEP-NET, but like in the case of breast cancer, studies are heterogeneous regarding cut-off levels and scoring techniques [21]. To our knowledge, GEP-NET is the only tumor type in which routine ki-67 staining is recommended. The mitotic count is a known prognostic factor in neuroendocrine lung tumors [23] which is a major criterion for subclassifications according the 2004 WHO classifications [24]. The mitotic count and ki-67 LI are both measurements of the proliferation rate, and ki-67 LI could potentially also be used in neuroendocrine lung tumors, yet no ki-67 LI cut-off point has been validated to correspond to the mitotic count.

Proliferating cells are generally considered more sensitive to antineoplastic treatments; as such agents often hamper the cell machinery during mitosis. Accordingly, the ki-67 LI may theoretically predict sensitivity to some chemotherapy agents as well [25]. Most studies concerning the predictive role of ki-67 LI has been performed in breast cancer patients. One study by Nishimura et al. found that pathological response after neo-adjuvant chemotherapy correlated with ki-67 values in multivariate analysis [26]. It was then suggested that patients with ki-67 above 25% could stratify to neoadjuvant chemotherapy [26]. The predictive role of ki-67 in adjuvant chemotherapy in breast cancer is rather inconclusive in suggesting a predictive role [27–29] or not [30].

The current article is a literature review to discover whether there is sufficient evidence of a prognostic and predictive role of ki-67 labeling index in NSCLC with focus on a possible role of histological subtypes and stages on the use of ki-67 LI. Also the cut-off level yielding the best prediction of outcome will be sought for.

2. Materials and methods

A literature search was performed in April 2012 using pubmed. Only papers published after 2000 were included to avoid significant heterogeneity caused by changes in staging and improved immunohistochemical staining methods. Keywords used were combinations of, "Ki-67, Ki67, MIB-1, proliferation marker, prognostic marker, predictive marker, NSCLC, Non-small cell lung cancer". References in selected articles were scrutinized and relevant articles were included in the review. Articles exploring ki-67 LI and correlation to prognosis or prediction of efficacy of systemic treatment and displaying a *p*-value were included. Articles in which data on NSCLC histology could not be extracted were excluded. Tables displaying included studies are presented (Tables 1–4).

3. Results

A total of 28 articles investigating the prognostic value of ki-67 in NSCLC fulfilled the inclusion criteria and were included. All studies were retrospective and varied considerable according to issues such as sample size, cut-off value levels, cut-off calculation methods, and clinical endpoints. Twenty-five studies included solely surgically treated patients stage I, I-II and I-III, respectively [31–54] while four studies also included inoperable stage IV patients [55–58].

There were severe variations regarding cut-off levels, which ranged from 5% to 30% ki-67 expressing cells. Statistical analysis was also done by comparison between several intervals or by defining ki-67 as a continuous variable in some papers. The rationale for the choice of cut-off values varied between the including papers. Thirteen papers did not explain how the cut-value was decided [31,33,34,42,44,45,47,49,53–55,57,58], three papers defined the median ki-67 labeling index value as cut-off point [32,36,41] and one study calculated and used the *H*-score median [52]. Seven studies referred to cut-off values used in previous articles [35,37–40,46,51], and one study used the best discriminatory value [48]. Two studies divided patients into 4 intervals according to ki-67 LI [50,56], while one study estimated a cut-off value that stratified patients into a group with low ki-67 LI with low variation in values and a group with high ki-67 LI with high variation in values [43].

All studied were rather consistent in assessing the ki-67 LI from an average of percentage of stained tumor cells throughout the tumor but the number of stained tumor cells varied. Most of the included papers counted at least 1000 cells were [31,33–35,38,39,41,43–45,48,50,58], while 500–1000 cells [32,47,59], 400 cells [51], 200 cells [46,53], and 100 cells [49] were counted in other studies, respectively. Eight studies did not mention the number of counted tumor cells [36,37,40,42,52,54,55,57].

3.1. Prognostic value of ki-67

3.1.1. Prognostic value of ki-67 according to percentage IHC positive cells

3.1.1.1. Ki-67 cut-off levels ≤10%. Five studies used a cut-off between high and low ki-67 labeling index from 5% to 10%. The aim of these papers was to evaluate the prognostic value of ki-67 after resection of tumor with curative intent. In four papers in which only stage I tumors were included, high ki-67 index was associated to either decreased overall survival or decreased disease-free survival in univariate analysis [31,46,47,53]. The agreement found between the studies in univariate analysis was not observed in a multivariate analysis by Yamashita et al. [46] who observed ki-67 LI to be prognostic regarding DFS while Inoue et al. [53] did not despite equal cut-off levels of 5%.

Carbognani et al. [54] included patients stage I–IIIA who also had been operated with curative intent and did not find any prognostic value of ki-67 LI in either uni- or multivariate analysis. All the above mentioned studies include a relatively small number of patients ($n = 100$) which hamper the statistical power of the studies.

3.1.1.2. Ki-67 cut-off levels 20–30%. Cut-off values of 20–30% distinguishing high versus low ki-67 LI were used in 18 studies. High ki-67 LI was a statistically significant negative prognostic factor regarding overall survival or DFS by univariate analysis in 7 studies [32,35,36,38,39,41,43,48,51] while it was not statistically significant in 8 studies [33,34,40,42,49,55,57,58]. In multivariate analysis, only 3 studies [32,35,43] observed ki-67 LI be a statistically significant prognostic factor while 9 studies did not [34,36,38–42,48,49].

3.1.2. Prognostic value of ki-67 according to disease stage

Five out of six studies including solely stage I disease patients [31,38,45–47] reported high ki-67 LI to be a negative prognostic factor while one was not statistically significant [34]. An adverse prognostic impact of high ki-67 LI was observed in 2 multivariate analyses [45,46] while 2 other studies [34,38] were not statistically significant.

The remaining included studies in the review includes several disease stages and as above, did not observe statistically significant impact of ki-67 neither in uni- nor multivariate analyses.

However, both Huang et al. [37] and Maddau et al. [51] performed subgroup analysis in the included patients staged I to III who were stratified according to a ki-67 LI cut-off level of 25%. Huang et al. observed that ki-67 was a significant prognostic factor