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Predictive role of erythrocyte macrocytosis during treatment with pemetrexed in advanced non-small cell lung cancer patients

Sebastiano Buti[a,*], Paola Bordi[a], Marcello Tiseo[a], Emilio Bria[b], Isabella Sperduti[c], Massimo Di Maio[d], Stefano Panni[e], Silvia Novello[f], Simonetta Grazia Rapetti[f], Sara Pilotto[b], Giovenzio Genestreti[g], Antonio Rossi[h], Debora Pezzuolo[i], Roberta Camisa[a], Giampaolo Tortora[b], Andrea Ardizzoni[a]

[a] Oncology Unit, University Hospital of Parma, Parma, Italy
[b] Oncology Unit, University of Verona, Azienda Ospedaliera Universitaria Integrata, Verona, Italy
[c] Biostatistics Unit, Regina Elena National Cancer Institute, Roma, Italy
[d] Clinical Trials Unit, Istituto Nazionale dei Tumori Fondazione 'G. Pascale' – IRCCS, Napoli, Italy
[e] Oncology Unit, Azienda Istituti Ospitalieri di Cremona, Cremona, Italy
[f] Department of Oncology, University of Turin, Azienda Ospedaliero-Universitaria "San Luigi Gonzaga", Orbassano, Torino, Italy
[g] Department of Oncology, AUSL Rimini, Cervesi Hospital, Cattolica (RN), Italy
[h] Division of Medical Oncology, S.G. Moscati Hospital, Avellino, Italy
[i] Oncology Day Hospital, Guastalla Hospital, AUSL of Reggio Emilia, Italy

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ABSTRACT

Objectives: Pemetrexed has been approved for the treatment of advanced non-small cell lung cancer (NSCLC) non-squamous histology, both as first- and second-line therapy. Pemetrexed is an antimetabolite drug, that inhibits enzymes involved in nucleotides bio-synthesis arresting cancer cells cycle. The aim of this study was the evaluation of the impact of pemetrexed on erythrocyte mean corpuscular volume (MCV) change and its possible correlation with disease control rate (DCR), progression free (PFS) and overall survival (OS) in NSCLC patients.
Materials and methods: A retrospective collection of clinical and laboratory data (including basal MCV and maximum MCV occurred during therapy) in advanced NSCLC patients treated with pemetrexed at seven Italian centers was performed. Nonparametric tests, univariate and multivariate analysis were used to assess correlation between variables and to identify predictors of outcomes.
Results: 191 patients were enrolled: median age 62, 60% male, 61% performance status (PS) 0, 91% stage IV, 88% adenocarcinoma histotype, 25% never smoker, 62% received pemetrexed as first-line. Mean MCV significantly increased from basal (89 fL) to during treatment (94 fL), with mean $\Delta MCV = 4$ fL. The median time from therapy start to maximum MCV was 2.2 months. Median PFS was 7 [CI95% 6–8] and 3 [CI95% 2–4] months [$P = 0.0016$], and median survival was 17 [CI95% 12–23] and 10 [CI95% 8–12] months [$P = 0.02$], in patients with $\Delta MCV > 5$ fL ($n = 80$) and $\Delta MCV \leq 5$ fL ($n = 111$), respectively. Multivariate analysis identified age $\geq$62, PS 0, adenocarcinoma histology and $\Delta MCV > 5$ fL as independent predictors of longer PFS. A $\Delta MCV > 5$ fL significantly correlates with DCR.
Conclusion: Pemetrexed induces macrocytosis. $\Delta MCV > 5$ fL on pemetrexed therapy correlated with better DCR, PFS and OS. These results deserve further validation in prospective studies.

1. Introduction

Lung cancer is still the leading cause of cancer related death in the world [1]. Non-small cell lung cancer (NSCLC) represents approximately 85% of all cases and is mostly diagnosed in advanced stage. In this setting, palliative platinum-based chemotherapy, is the only therapeutic approach for most patients, aiming to control symptoms and prolong survival [2–4].

* Corresponding author at: Oncology Unit, University Hospital of Parma, Viale Antonio Gramsci, 14, 43126 Parma, Italy. Tel.: +39 0521 702894; fax: +39 0521 995448.
E-mail address: sebabuti@libero.it (S. Buti).

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Pemetrexed has been licensed both in association with platinum and alone, as first and second line treatment, respectively, in patients with advanced non-squamous NSCLC [5–9]. Indeed, non-squamous histology represents the principal predictor of efficacy in NSCLC patients treated with pemetrexed. This evidence emerged from retrospective analyses and is probably related to low expression of thymidylate synthase (TS) enzyme, the principal target of pemetrexed activity [7–11]. However, TS expression does not seem to allow patient selection for pemetrexed therapy and, to date, validated predictive factors of pemetrexed efficacy in patients with non-squamous histology are still missing.

Pemetrexed is an antimetabolite drug interfering with enzymes involved in DNA synthesis. In particular, by inhibiting TS, dihydrofolate reductase (DHFR) and glycinamide ribonucleotide formyltransferase (GARFT), pemetrexed interferes with folate-dependent metabolic processes necessary to DNA replication and homocysteine homeostasis. Considering that pemetrexed is non target specific, the effects of its activity involve also other tissues than lung cancer, such as bone marrow. In 2002 a correlation between higher pemetrexed toxicity and folate deficiency, as expressed indirectly by high homocysteine blood levels, was identified [12]. Since then, concomitant implementation with folic acid and B_{12} vitamin has been recommended during pemetrexed treatment, resulting in reduced toxicity without compromising efficacy [13,14].

Methylenetetrahydrofolate reductase (MTHFR) is another enzyme involved in folate metabolism and catalyzes the synthesis of l-5-methyltetrahydrofolate, essential in DNA synthesis and homocysteine metabolism [15,16]. Polymorphisms of MTHFR have been reported in the literature, resulting in low enzymatic activity [17] potentially able to predict survival differences in pemetrexed-treated NSCLC [18]. Patients undergoing pemetrexed therapy are given prophylactic vitamin supplementation with folic acid and B_{12} vitamin as indicated, therefore a folate deficiency can be excluded [19]. However, the conversion of folic acid synthetic form is inhibited by the activity of pemetrexed on DHFR. Therefore, despite supplementation, pemetrexed could reduce the amount of substrate available for MTHFR activity revealing a possible condition of low enzymatic activity, due to polymorphism [16]. Finally, in bone marrow, this could affect normal erythropoiesis and result in increased red cell MCV. Hence, MCV growth could be indirect expression of the MTHFR polymorphisms presence, identifying a subgroup of patients particularly sensitive to pemetrexed in terms of both toxicity and activity.

The correlation between MCV and antimetabolite drugs has been previously reported in a group of patients treated with capecitabine [20,21]. Moreover, some data suggest that, in women with metastatic breast cancer, MCV elevation while on capecitabine could represent a response predicting factor [22,23].

We designed this retrospective multicentric study in a population of advanced NSCLC treated with pemetrexed, to identify changes in MCV value during treatment and their possible role as predictive and prognostic factor.

2. Materials and methods

2.1. Study population

This was a retrospective, observational multicenter study of medical records from October 2007 to February 2012 for consecutive patients with advanced NSCLC treated with chemotherapy at seven different centers in Italy. Data were retrospectively collected from medical chart reviews and electronic records. Inclusion criteria were: patients of all ages who received intravenous chemotherapy with cisplatin/carboplatin in combination with pemetrexed or pemetrexed alone (in accordance with their treating physician's practice), histologic or cytologic diagnosis of inoperable NSCLC (any histology), administration of at least one dose of pemetrexed, availability of laboratory and clinical data from the baseline to at least one month after the end of chemotherapy, written informed consent (for alive patients). Our study included also patients with squamous NSCLC histology and treated with pemetrexed before 2009, when the differential efficacy of pemetrexed according to NSCLC histology was not yet known [24]. Patients were excluded from the analysis if they had missing key information (i.e. laboratory data, clinical assessment, survival data) or other cancer treated with pemetrexed-based chemotherapy (i.e. pleural mesothelioma).

Patient characteristics and clinic-pathological variables considered in this study were: gender, age, Eastern Cooperative Oncology Group (ECOG) performance status (PS), previous chemotherapy, stage, histological subtype, smoking history, MCV value within one week before the start of pemetrexed chemotherapy, maximum MCV value (and its date) obtained during the time ranging from the day after the first cycle and one month after the last cycle ("during treatment" MCV), concurrent administration of platinum, toxicity and objective response. In particular tumor response was determined by the investigators on the basis of the Response Evaluation Criteria in Solid Tumors 1.1 [25] every 3 cycles of treatment (or before in the case of clinically suspected disease progression); complete blood counts with MCV was obtained at each chemotherapy cycle and toxicity was graded using the National Cancer Institute Common Toxicity Criteria Version 4.0 [26].

This study was approved by the ethical committees and review boards of the institutions included in this analysis.

2.2. Statistics

Descriptive statistics was used to summarize pertinent study information. Follow-up was analyzed and reported according to Shuster [27]. The correlation between variables was analyzed according to chi-square, Student's *t*, and Mann–Whitney (non-parametric) tests. The hazard ratio (HR) and the 95% confidence intervals (95% CI) were estimated for each variable using the Cox univariate model [28,29]. A multivariate Cox proportional hazard model was developed using stepwise regression (forward selection, enter/remove limits $P = 0.10$ and $P = 0.15$), to identify independent predictors of outcomes. The receiver operating characteristic (ROC) curve analysis was adopted for dichotomization of continuous variables according to outcome [30]. Progression free survival (PFS) and overall survival (OS) were calculated by the Kaplan–Meier product limit method from the date of treatment start until progression or death for any cause [31]. The log-rank test was adopted to assess differences between those variables resulted to be independent at multivariate analysis. Significance was defined at the $P < 0.05$ level. The SPSS® (18.0), and MedCalc® (10.0.1) licenced statistical programs were used for all analyses.

3. Results

3.1. Patients

Overall, 191 patients were included in our analysis (Table 1); 60% of patients were men, the median age was 62 years (range 36–83), 61% were ECOG PS 0, 91% were advanced (stage IV), 88% were adenocarcinoma histotype, and 75% were current or ex-smokers. Pemetrexed was administered as first chemotherapy in 62% of patients; combined with a platinum compound in 72%; the median of administered cycles was 4. All patients received vitamin B_{12} and folic acid supplementation. 94% of patients presented with normal basal MCV (<97 fL). The mean MCV increased from pretreatment