RED CELL DISTRIBUTION WIDTH (RDW) - A POTENTIAL PROGNOSIS FACTOR IN MALIGNANT MELANOMA

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RED CELL DISTRIBUTION WIDTH (RDW) - A POTENTIAL PROGNOSIS FACTOR IN MALIGNANT MELANOMA (Abstract):Although malignant melanoma accounts for less than 5% of all cancers, it is one of the most aggressive types of cancer, associated with a poor outcome. Survival rates significantly vary according to stage and even within the same stage there are significant survival differences. The aim of this study was to assess the role of red cell distribution width (RDW) as a potential prognosis factor for malignant melanoma. Also, this analysis aims to determine average survival and the time until relapse/progression of the disease in patients with malignant melanoma treated at the Regional Institute of Oncology Iasi (IRO) between 2013 and 2016. Materials and methods: We performed a retrospective analysis of melanoma patients treated in the IRO between 2013 and 2016. We collected several parameters including age, sex, tumor localization, clinical and pathological stage, Breslow Index, Clark level, ulceration and detailed therapeutic management. We then analyzed each parameter to assess its impact on progression-free survival and overall survival. Results: We identified RDW as a significant prognosis marker, with worse outcome for patients with abnormal RDW values. Additionally, tumor localization, Clark level, Breslow index and cancer stage also showed prognosis value. Conclusions: RDW is a useful, inexpensive prognosis marker that can better predict survival. Prospective studies are required for confirming the findings of the present analysis. Keywords: MELANOMA, RED CELL DISTRIBUTION WIDTH, PROGRESSION FREE-SURVIVAL, OVERALL SURVIVAL.

Along with the worldwide population ageing trend (1), there is a progressive increase in the incidence of a large number of cancers, such as colon cancer, hepatocarcinoma (2), breast cancer and melanoma (3). Although malignant melanoma currently represents less than 5% of cancers, it is estimated that in 2014 this neoplasia was responsible for over 9,000 deaths in the United States alone (4), being considered one of the most aggressive types of cancer and generally associated with a bad prognosis (5). Furthermore, epidemiological reports from recent years have shown there is a tendency towards the decrease of the median age of diagnosis, together with a significant increase in melanoma incidence in males (6). In Europe, the countries with the highest number of melanoma cases are in Germany, Switzerland, Norway, Denmark and Iceland (5). In Romania, melanoma is the second most frequent type of
cancer in individuals aged 15-29 years, with an increase rate of 4% per year (7).

Traditionally, malignant melanoma is believed to be a cancer associated with an unfavorable outcome. With the improvement of surgical and radiotherapy techniques, along with the introduction of immune therapy and the systematic testing of the BRAF mutation, new therapeutic approaches have significantly improved the quality and life expectancy of patients with metastatic malignant melanoma (8). Five-year survival is estimated to be 62% for patients with lymph node invasion at diagnosis and 18% for those diagnosed directly in the metastatic stage (9), results which are now well above the epidemiological reports of previous decades. However, survival assessment remains difficult (10) and there are significant variations within the same disease stage (11). Also, similar to other types of tumors (12), there are certain clinical situations where diagnosis and staging are difficult due to lack of symptom specificity and aggressiveness of the disease. In addition to TNM staging (tumor size, lymph node invasion, and presence of metastasis) recommended by the American Committee Against Cancer (AJCC), which remains the most prevalent method of assessing prognosis (13) for many cancer types (14), there are certain "classical" prognosis factors considered to have an independent impact on survival in malignant melanoma. Of these, male gender, advanced age, localization on the head, neck or trunk, increased Breslow and Clark levels, presence of ulceration, and increased mitotic rate (15) are associated with a more bleak prognosis, regardless of the stage at diagnosis.

Together with a significant increase in the number of preclinical and clinical cancer studies (16) that aim to improve diagnosis or find new methods of treatment (17), more and more new potential prognosis factors have been assessed which has led to the identification of some parameters that can predict significant modifications of the length of survival and have a predictive role in the response to treatment. Some of these, such as the presence of BRAF mutation, sentinel lymph node status, LDH level or S100 protein, are already used in the clinical setting (18), whereas others such as vitamin D (19), lymphocyte infiltration (20), or genetic modifications identified by means of "next generation sequencing" (NGS) techniques (11) have not yet gathered sufficient scientific evidence or cannot be implemented on a large scale due to significant additional costs. As such, there is still a need to identify new prognostic factors that are easy to assess for malignant melanoma to individualize patient prognosis.

Red cell distribution width (RDW) is a commonly-assessed parameter included automatically in the complete blood count of patients; it determines the presence of variations in the size or volume of red blood cells (21). In recent years, it has been assessed as a prognosis factor in several types of solid tumors such as lung (22), prostate (23), endometrial (24) or hepatic (25) cancer and it has been identified as a relevant prognosis factor for all these localizations. However, to the authors' knowledge, RDW has not been previously assessed as a prognostic factor for patients diagnosed with malignant melanoma.

The aim of this paper is to assess the role of RDW as a potential prognosis factor for malignant melanoma. Also, this analysis aims to determine average survival and the time until relapse/progression of the
disease in patients with malignant melanoma treated at the Regional Institute of Oncology Iasi between 2013 and 2016. One other aim is to confirm the value of established prognostic factors in assessing survival for patients included in the present analysis.

**MATERIAL AND METHODS**

We performed a retrospective analysis on all cases of cutaneous melanoma treated at the Regional Institute of Oncology (IRO) Iași between 2013 and 2016. IRO is a tertiary medical unit specialized in diagnosing and treating both hematological and solid malignancies that serves as a reference center for Moldova, the north-eastern region of Romania. In IRO, melanoma diagnosis is based on pathology assessment of tissue and staging is determined based on CT, MRI, or PET-CT imaging studies. All cancers diagnosed in IRO are staged according to AJCC’s TNM system. Performance status is routinely assessed by means of the World Health Organization approved Eastern Cooperative Oncology Group (ECOG) PS scale.

Inclusion criteria for the present analysis were histological confirmation of cutaneous melanoma (incisional/excisional biopsy or surgery), receiving local or systemic treatment in IRO, a performance status ≤ 2 at the time of the diagnosis and being over 18 years of age.

Exclusion criteria were: a history of a prior malignancy (non-melanoma skin cancer excluded), chemo/radiotherapy prior to the initiation of melanoma treatment, non-cutaneous melanoma localization, doing treatment and follow-up in another medical unit and performance status > 2.

Patient records were accessed by means of the hospital’s electronic database. We collected a series of patient’s characteristics, tumor and clinical data for each case. Parameters were structured as follows: demographic data: sex, age, urban/rural residence, clinical data: tumor localization, clinical TNM stage, presence and localization of metastasis, ECOG performance status, biochemical data (before treatment): LDH, RDW, platelet distribution width (PDW), white blood cell count, hemoglobin, histology and molecular biology data: Clark level, Breslow index, presence/absence of ulceration, pathology TNM stage, S100, BRAF, treatment data: surgery, radiotherapy, first-line and second-line anti-cancer systemic treatment, toxicity of treatment, survival data: date of diagnosis, recurrence date (if applicable), overall survival.

Statistical evaluation was performed using SPSS version 20.0 software. Unpaired Student’s *t*-test, Chi-squared, Kaplan-Meyer survival curve and Fischer exact test were used according to data type. For each collected parameter, we assessed its potential impact on overall survival. Statistical significance was defined as *p* < 0.05; variables were considered independent for the statistical analysis; continuous data was expressed as mean ± standard error.

**RESULTS**

**Descriptive analysis**

Of the 120 patients identified in the electronic database, 75% of the patients fulfilled the inclusion criteria. 25% of the patients were not included in the analysis because they had non-cutaneous melanoma or because they either refused treatment or received it in a different hospital.

In our study group, the male: female ratio was 1.04:1, with 51.1% male and 48.9% female. Average age at diagnosis was 58.56...
years, with a median of 59 years. Most patients were younger than 65 at diagnosis (61 out of the 90 patients) and lived in the urban area (74.4%). Regarding localization, most primary tumors were in the lower limbs (32.2%), followed by posterior thorax (22.2%), anterior thorax (12.2%) and abdomen (11.1%). From a clinical staging point of view, most patients were diagnosed in stage II disease, with 18 patients being diagnosed in stage IIA, 13 in stage IIB and 12 in stage IIC. The rest were most often diagnosed in stage III (27.7% patients) or stage IV (7.77% patients) of the disease. Only four patients were diagnosed in stage I. Most frequent metastatic sites included brain, lung, liver and bone.

Regarding performance status, most patients (91.1%) had ECOG = 1 at diagnosis, whereas 4.44% were ECOG = 0 and 4.44% were ECOG = 2. Biochemical assessment before treatment initiation indicated that 38.9% of the patients had increased LDH levels, 36.6% had lymphocytosis, 13.3% had a decreased monocyte count, and 24.4% had grade I or II anemia.

Pathology and molecular biology analysis of the biopsy/surgical piece revealed that most melanomas had a Breslow index of over 4 mm (44.4%) and only 2 patients had a Breslow index < 1 mm. Similarly, Clark levels were most often IV (27%) or V (25%), with only one patient being assessed as a Clark I. More than half of the patients (56.7%) presented ulceration at diagnosis and most cases were upstaged after tissue analysis (cTNM vs. pTNM). Regarding BRAF mutation, it was only tested in 15 patients, 6 of which were found to be BRAF positive. S100B protein was increased in 18.8% of patients, with normal levels in 74 patients.

Regarding treatment data, most of the patients underwent surgery as a first-line localized therapy option. Throughout the course of the disease, however, almost all patients underwent curative or palliative surgery and most of them benefited more than once from this approach (96.6% of patients with 152 surgical procedures performed). Adjuvant therapy included interferon in three cases and radiotherapy if positive margins, although most localized melanomas were simply followed-up periodically. Most patients in stage II or III disease quickly developed local or generalized recurrences and received either local therapy (second surgery and/or radiotherapy) or first-line systemic therapy. Radiotherapy was recommended in 23.2% of patients, either at the primary site (12.2% of patients) or for brain metastases (11.1% of patients). Dacarbazine was the preferred first-line chemotherapy choice in all cases. Second-line chemotherapy options were Carboplatin (10%), Cisplatin and Paclitaxel (4.44%) or Temozolamide (2.22%). One in four patients (24%) could not complete the projected number of cycles due to unacceptable drug toxicity. One patient refused systemic treatment after being diagnosed with systemic recurrence six months after surgery.

Maximum progression-free survival recorded was 191 months; survival time had a minimum of one month and an average of 42 months. Average progression-free survival was 22.46 months.

**Prognosis factors**

After analyzing the potential relevance as a prognosis factor, five of the assessed parameters were found to be associated with prognostic variations: primary tumor localization, Breslow index, Clark level, stage and RDW values.
Primary tumor localization was associated with a better outcome if the tumors were in the limbs when compared with abdominal or thoracic melanoma (p=0.043 for limb vs. abdominal melanoma and p=0.036 and p = 0.021 for limb melanoma versus anterior and posterior thoracic melanoma). Average survival for a patient with melanoma on the abdominal cutaneous surface was 29 months and 26 months for thoracic melanoma. In contrast, lower-limb melanoma patients had an average survival of 36 months and upper-limb melanoma patients had an average survival of 77 months, a statistically significant difference.

Breslow index was found to be a significant prognosis factor, with better outcome for a Breslow < 1 mm when compared with a Breslow > 2.1 mm (p=0.008). Recurrence-free interval was 142 months for patients with Breslow < 1 and progression-free survival (PFS) abruptly decreases as the Breslow index is higher, with a 48-months value for a 1-2 mm Breslow index. Similarly, Clark level also showed prognosis value, with statistically different survival averages between level III when compared with level IV and level V (p=0.006, p=0.010). Progression-free survival was 142 months for Clark I, with a progressive decrease of survival as Clark levels grow (23 months overall survival for Clark V).

In our analysis, stage had a significant prognosis value. Patients with clinically noticeable lymph node invasion (N = 33) had a worse outcome when compared with patients with negative lymph nodes (p<0.016). Among those without node involvement at diagnosis, 42.2% quickly presented with local or systemic relapse. Average survival was 21 months for patients with lymph node involvement and 46 months for those without. Patients diagnosed directly with stage IV disease had a worse prognosis when compared with localized or locally advanced disease (p=0.00038), with an average survival of seven months for metastatic disease. Stage had a statistically significant impact on survival, with stage IV patients that were associated with worse prognosis when compared with other stages (p=0.006, p=0.016, p=0.013 when compared with stage I, stage II and stage III disease).

RDW also had a significant prognosis value - patients with an RDW > 15% (increased) had a decreased survival time when compared with those with normal RDW values at diagnosis (p=0.030). Average survival was 33 months for patients with abnormal RDW values compared with 64.97 months for those with normal RDW values (fig. 1).

Several other factors were assessed in this analysis and showed little or no influence on prognosis. Although a small benefit was noted for females, distribution according to sex did not show statistically significant differences. Similarly, age distribution, LDH and S100 values were not shown to have prognosis values. A small benefit of survival (38 vs. 37 months) was shown for patients without tumor ulceration, but it don’t reach statistical significance. Since generalized BRAF testing was only available in IRO in 2015, we could not assess the effects of BRAF mutations on survival. All patients in non-metastatic stages received surgery and first-line chemotherapy was in all cases Dacarbazine, so no comparison could be performed. Second-line chemotherapy was not initiated in all cases and no statistical differences were found between treatment protocols.
DISCUSSION

The present retrospective study included 90 patients who received treatment in IRO Iasi. The maximum survival rate registered in this group was 191 months, with a mean survival of 42 months; progression-free survival was an average of 22.46 months. Gender did not have a significant influence on overall survival, although we noticed a numerical difference in favor of the female sex. Literature data is not consistent regarding the influence of gender on survival rate, although a study assessing 3,900 melanoma patients showed that women appear to have a better prognosis than males due to a protective factor that was not yet clearly identified (26). Our analysis did not identify a difference in gender survival, most likely due to the small number of patients included.

Regarding age as a prognostic factor, we did not identify any significant differences between patients. This is somewhat surprising, taking into account the fact that elderly have a tendency for self-neglect (27) and often have cognitive dysfunctions and other relevant comorbidities that can significantly delay and influence both diagnosis and treatment (28,29). However, we have interpreted this result because of patient selection - there is an inherent bias to choosing only patients that choose to receive treatment in a cancer institute and are fit enough to do so.

Fig. 1. Patient survival and RDW values before treatment initiation
Red cell distribution width (RDW) – a potential prognosis factor in malignant melanoma

Similarly, the place of residence (urban versus rural dwellings) did not have a significant influence on survival, a result which is different from other literature data. According to several retrospective studies conducted in Canada, there was a higher incidence of melanoma reported in urban vs. rural areas. Researchers explained this by pointing out that people living in a city have easier access to see a dermatologist, which increases the chance of diagnosing atypical nevi with malignant potential and diagnosing melanoma in general, concluding that actually there is no real difference in prevalence in urban versus rural areas (30).

Primary anatomical localization was identified as a major prognosis factor, with significant statistical differences between melanoma of the extremities versus melanoma located on the abdomen and on the anterior and posterior thorax. In this analysis, we noted a significant survival advantage for patients diagnosed with melanoma in the limbs. These data is consistent with other literature data, considering that in a study of 2,500 patients, melanoma located on the neck, trunk and head had worse prognosis when compared with other sites (31).

Stratifying according to the Breslow Index showed a significant difference in survival rate, with longer survival for less invasive melanomas. An increase in the Breslow Index was associated with a worse outcome, with a steep decrease in recurrence-free survival for a 1-2 mm Breslow (48 months). According to the AJCC, in a study of 1,841 patients, the 10-year overall survival rate was 92% for Breslow Index 1, 80% for Breslow Index 2, 63% for Breslow Index 3 and 50% for patients with the Breslow Index 4, consistent with the results of this analysis (32). Similar results were observed for the Clark level, with a progressively decreasing overall survival from Clark I (142 months) to Clark V (23 months).

Our data indicated the TNM stage was another relevant prognostic factor, with a significant increase in the risk melanoma recurrence or progression for those patients with positive lymph nodes at the time of diagnosis. Thus, average survival was 21 months for those with positive lymph nodes and 46 months for those with negative lymph nodes, regardless of the stage of the disease. According to literature data, lymph node invasion is a well-established prognostic factor with studies reporting that negative lymph node patients have a 76-90% PFS at 5 years, whereas those with positive lymph nodes have a PFS of 35-58% at 5 years (33). The presence of systemic disease at diagnosis is considered an independent prognosis factor with a mean survival of one year for stage IV patients according to one study (34). Our results fall in line with available data - in IRO, the survival of patients with metastatic malignant melanoma was 7 months.

RDW variations were associated with different outcomes. There was a statistically significant difference in survival for patients who had RDW above 15% (increased) when compared with those with normal RDW values. Average survival was 33 months for patients with increased RDW and 64.97 months for those with normal RDW prior to treatment initiation. In recent years, RDW has become an attractive prognosis factors and has been assessed in several types of tumors, showing that it can be used as an independent means for predicting survival. In a study of over 300 patients, Koma&colab. found RDW to accurately predict survival in lung cancer, not only for advanced, but also for localized stages of the disease (22). RDW was found to predict survival of patients
with pancreatic cancer in a study conducted in Turkey on a group of 104 patients (35). Also, in a Chinese study performed on a group of 608 patients, abnormal RDW values were correlated with a worse outcome in breast cancer patients (36).

Due to the characteristics of the present analysis, several factors considered predictive by international guidelines did not have any effect on survival. Thus, a definite correlation could not be made between the presence of ulceration and patient survival due to incomplete pathological staging (in some cases) and the small group of patients. In a study performed on a group of 423 patients in Spain, ulceration was significantly correlated with a decrease in both overall survival and PFS survival (37). Since routine BRAF mutation testing has been only recently introduced in IRO standards, the number of patients tested for BRAF mutation was too small to achieve a definite correlation. Also, we did not record significant differences in overall survival when stratifying by LDH or S100b protein level. A possible explanation for these findings would be reduced specificity of these markers (abnormal levels are also found in patients with hepatic, renal, various inflammatory and infectious diseases). Also, in this analysis we included patients diagnosed in all disease stages, whereas most studies that indicate these markers as prognosis factors were performed on stage IV patients. In a study on a group of 855 patients with melanoma, both LDH and S100b values were classified as independent prognostic factors for metastatic melanoma (38). However, other studies on these two markers were inconclusive (39).

The general research trend encourages and supports identifying new treatment methods, molecular biology or genetic risk factors and funds the development of novel drugs. However, some of the clinically used drugs and some of the already available markers might have surprising uses in different practical settings (40). Changing drug ratio for active combinations (41) or simply reanalyzing some of the markers at hand can lead to novel results and improve daily practice without additional costs.

**Limits of the study**

The design of this analysis is retrospective, and it was conducted in a single oncology institute. The number of patients included in the study was quite small (90 patients) and several types of analysis were performed irrespective of the patient’s stage. We were not able to confirm several of the well-known prognosis factors for melanoma.

**CONCLUSIONS**

In our study, red cell distribution width is a promising new prognosis factor for patients with malignant melanoma. Alongside other parameters already in use, regularly assessing it can improve survival predictions and aid physicians working with cancer patients in decision-making and communicating with the family. Additional studies, preferably with a prospective design, are required to certify our analysis’ findings.

**REFERENCES**

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