

The discriminative properties of erythrocyte anisocytosis in patients with resectable malignant pancreatic masses compared with an age and gender matched control group

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SUMMARY: The discriminative properties of erythrocyte anisocytosis in patients with resectable malignant pancreatic masses compared with an age and gender matched control group.

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Introduction. Red cell distribution width (RDW) is a parameter that reflects the heterogeneity in the size of the circulating erythrocytes. Elevated levels of RDW have been found related to various pathologic conditions including cancers. We hypothesized that elevated RDW levels might correspond to advanced stages of pancreatic cancer.

Methods. The medical files and pathology reports of patients who underwent Whipple procedure were evaluated in a retrospective case-control study. The relationship of RDW to age, gender, cancer antigen 19-9 (CA 19-9), blood hemoglobin levels, stage of the tumor, tumor size, tumor grade, number of lymph nodes with metastasis, the status of lymphovascular and perineural invasion were investigated. A one-to-two, age- and- gender matched group of seemingly healthy patients was used to compare the levels of RDW between the patients of

pancreatic head malignancy and healthy people. ROC (receiver operating characteristics) curves analysis was conducted to define a threshold to discriminate malignant pancreatic head tumors from healthy control subjects. Then, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR), and negative likelihood ratio (-LR) were calculated.

Results. The median age was 61 years (IRQ 54.75-69). The median RDWs were 15.20% (IQR 13.20-19.50) vs 13.00 (IQR 12.60-13.48) in the pancreas adenocancer and the control group, respectively, $p=0.000$). There was significant relationship between RDW and the grade of the tumor, $p=0.026$. An RDW level of 14% was obtained as a result of the ROC curves analysis and the positive predictivity about malignancy was 70.73% with a negative predictive rate of 89.95% compared with the healthy group of patients.

Conclusion. The elevated levels of RDW in patients with a pancreatic mass may point to a possible malignancy; however, an elevated RDW in a patient with a malignant pancreatic head mass is related to the grade of the disease, not the stage.

KEY WORDS: Pancreatic cancer - Red cell distribution width - Predictivity

Introduction

Red blood cell distribution width (RDW) is one of the parameters routinely reported in the complete blood cell count test. It is a measure of the degree of the variability in size of mature erythrocytes in peripheral blood (1). This parameter has been used to discriminate the patients with iron deficiency anemia and thalassemia traits for a long time (2). Higher RDW values, which reflect greater variation in the size of red blood cells,

are found to be related to inflammation, malnutrition and impaired renal function, probably reflecting an insufficient amount of erythropoietin production (3). RDW has been investigated actively in the recent period and was reported to be possibly linked to a variety of disorders. RDW has been found to be related to the presence of chronic diseases such as gastrointestinal and liver diseases, as well as being associated with all-cause mortality and death from cancer, cardiovascular, and respiratory diseases in general population (4). A growing number of studies have shown the parameter to be an independent prognostic factor in lung and prostate cancers and chronic lymphocytic leukemia (5-8).

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Inflammation is reportedly an important factor in cancer (9). Growing amounts of data have reported that inflammation is an important component of the tumor progression; early in the neoplastic process, especially, inflammatory cells are powerful tumor promoters, making an attractive environment for tumor growth, enhancing genomic instability, and promoting angiogenesis (10).

Having a dismal prognosis, pancreatic cancer is ranked as the 14th most common cancer and is the 7th highest cause of cancer mortality in the world (11). RDW has been used as a parameter to discriminate malignant diseases, such as a fibroadenoma from breast cancer and in differentiating benign and malignant causes of obstructive jaundice (12, 13). The relationship of RDW to pancreatic cancer is a subject of interest.

We investigated the discriminative properties of RDW in pancreatic adenocarcinoma patients in comparison to an age-and-gender matched group of seemingly healthy individuals. Our hypothesis was that RDW levels might be associated with cancer progression: an increase in RDW might correspond to higher stages of the disease. Our primary outcome was the relationship of RDW to the TNM stages of pancreatic adenocarcinoma (14).

Patients and methods

The study was approved by the Haseki Training and Research Hospital's institutional review board with the number of 120/18/04/2018. Patients who underwent Whipple procedure because of a pancreatic adenocarcinoma between May 2014 and May 2018 were reviewed. The diagnosis of pancreas cancer was rendered postoperatively by checking the pathology reports of the surgically resected specimen. Blood samples were collected within one month prior to the operation, during routine preoperative preparation and diagnostic period. The normal RDW level in our laboratory is 11,5% to 14,5%. To evaluate the difference between healthy patients and the cancer group, a one-to-two, age-and-gender matched control group was constructed. Sixty-eight patients were selected randomly from a group of seemingly healthy patients of the American Society of Anesthesiologists (ASA) class 1-2 (15), who underwent repair for inguinal

hernias.

Normality was assessed by Shapiro-Wilk goodness-of-fit test. A P-value <0.05 was considered statistically significant. Non-normally distributed data were displayed as median and interquartile range (IQR), and normal data were displayed as means and standard deviations (SDs). Univariate and multivariate analyses were conducted between RDW and age, gender, cancer antigen 19-9 (CA 19-9), blood hemoglobin levels, stage, tumor size, tumor grade, number of lymph nodes with metastasis, the status of lymphovascular and perineural invasion.

The difference between RDW and hemoglobin levels between the pancreas cancer group and the control group were analyzed by Mann-Whitney U test. To measure the prognostic performance of the RDW to discriminate between cancer and healthy patients using receiver operating characteristic curves (ROC) and calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR), and negative likelihood ratio (-LR) for different cutoff values. We used JASP 0.920 (University of Amsterdam), and IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

Results

A total of 54 Whipple (Pancreaticoduodenectomy) operations were carried out between May 2014 and May 2018. Of these patients, 37 had underwent Whipple procedure because of presumably malignant pancreatic masses in the head (Figure 1).

On the basis the final pathology reports, a total of 3 cases (chronic pancreatitis, neuroendocrine tumor, and borderline mucinous tumor) were excluded. Therefore, 34 patients with pancreatic adenocancer who had underwent curative surgery and surgical staging were included (Table 1). The data were tested for normality and found to be non-normally distributed and, thus reported as medians and IQRs. The median age was 61 years (IRQ 54.75-69) in both groups. The median hemoglobin levels in the groups were 11.50 g/dL (IRQ 10.35-12.93) vs 14.4g/dL (IRQ 13.28-15.70), respectively. The median RDWs were 15.20% (IQR 13.20-19.50) vs 13.00 (IQR 12.60-13.48) in the pancreatic adenocancer and the control group, (p=0.000 and p=0.000, respectively).

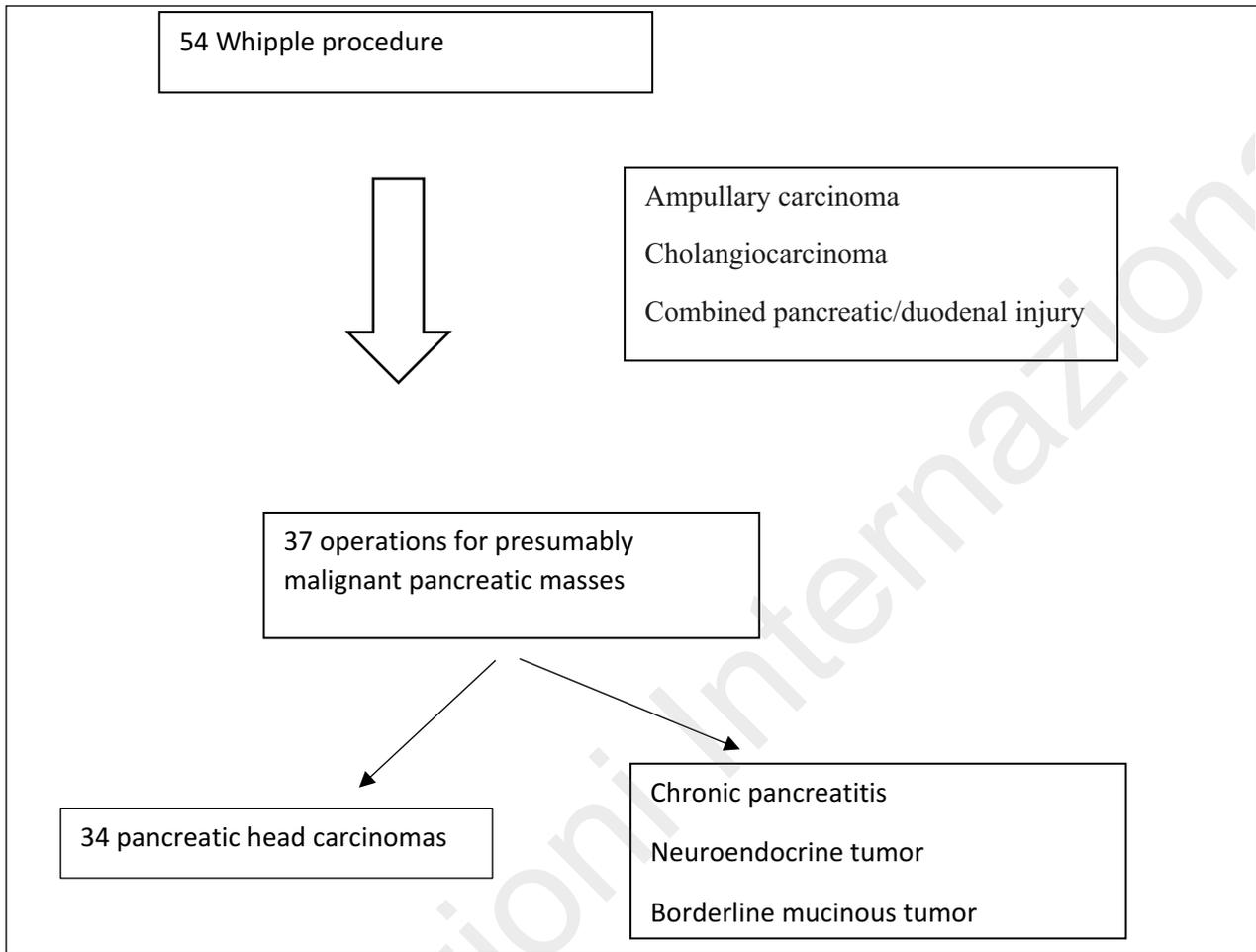


Figure 1 - Flowchart.

TABLE 1 - THE DESCRIPTIVE STATISTICS OF THE PANCREAS CANCER GROUP.

N:34 patients	Mean	Median	Minimum	Maximum	25th percentile	75th percentile
Age	60.7	61.0	29	75	54.7	69.0
Ca19.9	1077.0	108.2	8.2	1444.0	14.7	1164.0
Hbg	11.67	11.50	8900	16.30	10.35	12.93
RDW	15.37	15.20	13.20	19.50	14.28	16.25
Tm	3.13	3.25	0.50	5.00	2.00	4.00
N	1	1	0	13	0	2

Age in years, CA 19-9 (cancer antigen 19-9) in U/ml, Hbg: hemoglobin g/dL RDW: red cell distribution width in percentages, Tm: tumor size in centimeters, N: the number of metastatic lymph nodes.

Univariate analysis showed no significant relationship of RDW with age, CA 19-9 levels, the stage, tumor size, number of lymph nodes with metastasis, the status of lymphovascular and neural invasion but with blood

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hemoglobin levels and the grade of the tumor (Table 2). RDW levels did not differ between the male and the females 15.20 (IQR 14.05-16.13) *vs* 15.10 (IQR 13.80-16.70), respectively (p=0.916). Then a multivariate analysis was conducted. The only significant relationship was between RDW and grade (P=0.021) (Table 3). Receiver operating characteristic curves were plotted for RDW to identify patients of pancreatic cancer compared with a group of

gender-and-age matched otherwise healthy patients who underwent hernia correction, with a statistically significant area under the curve of 0,881 (95% confidence interval, 0.809-0.952) (Figure 1). We also calculated the PPV, NPV, +LR, and -LR for RDW at a cut-off value of 14.0% which predicted malignancy in 70% of patients (Table 4). Kruskal Wallis test showed no relationship of RDW to the stage, p=0.446

TABLE 2 - RDW IN UNIVARIATE ANALYSIS.

	Age	Stage	CA19-9	Hbg	Tm size	Grade	N	LFI	PNI
P value	0.625	0.916	0.844	0.032*	0,435	0.021*	0.318	0.785	0.639

Age in years, CA 19-9 (cancer antigen 19-9) in U/ml, Hbg: hemoglobin g/dL, RDW: red cell distribution width in percentages, Tm: tumor size in centimeters, N: the number of metastatic lymph nodes, grade of the tumor 1-3, LFI: presence of the lymphovascular invasion of the tumor, PNI: presence of the perineural invasion. * significant at level P<0.05.

TABLE 3 - THE SIGNIFICANCE OF RDW IN A MULTIPLE REGRESSION MODEL.

	Age	Stage	Ca19-9	Hbg	Tm size	Grade	N	LFI	PNI
P-value	0.552	0.704	0.758	0.078	0.326	0.026*	0.537	0.979	0.951

Age in years, CA 19-9 (cancer antigen 19-9) in U/ml, Hg: hemoglobin g/dL, RDW: red cell distribution width in percentages, Tm: tumor size in centimeters, N: the number of metastatic lymph nodes, grade of the tumor 1-3, LFI: presence of the lymphovascular invasion of the tumor, PNI: presence of the perineural invasion. * significant at level P<0.05.

TABLE 4 - SENSITIVITY, SPECIFICITY, POSITIVE LIKELIHOOD (+LR) AND NEGATIVE LIKELIHOOD (-LR), POSITIVE PREDICTIVE VALUE, NEGATIVE PREDICTIVE VALUE OF RDW FOR MALIGNANCY AT OPTIMAL CUT-OFF.

RDW	Sensitivity (95% CI)	Specificity (95% CI)	+LR	-LR	Positive predictive Value (95% CI)	Negative predictive Value (95% CI)
>14.00	80.56 (63.98- 91.81)	83.33 (72.70-87.81)	4,83 (2.81-8.30)	0,23 (0.12-0.46)	70.73 (58.46-80.58)	89.55 (73.90- 89.06)

Discussion

RDW levels have been found to be elevated in pancreatic adenocarcinoma patients when compared with a control group of seemingly healthy patients with age-and-gender match. In the multivariate analysis RDW level was only found to be significantly related with the grade of the cancer, but not with tumor stage. RDW has been subject to various studies, investigating its relationship to cancers. Pancreatic cancer is a major cause of cancer-associated mortality, with a dismal overall prognosis that has remained almost the same for many years.

Currently, prevention or early diagnosis at a curable stage is exceedingly difficult; patients rarely exhibit symptoms and tumors do not display sensitive and specific markers to aid detection (16). This study has been designed to see whether RDW might be employed as a tumor marker in pancreatic cancer, since cancer associated-inflammation has been found related to tumor genesis and progression (17). In a study of patients with ovarian cancer the investigators observed significant difference in RDW level among four stages of ovarian cancer (18). The RDW levels increased as the stage of the cancer advanced. In the same study they found out that the cancer antigen 125 (CA-125) concentration was remarkably increased in the ovarian cancer group, and the RDW levels were positively correlated with the rise in the CA-125 concentration.

Huang et al. (19) studied the RDW in breast cancer patients younger than 40 years of age. In this study, they found that high RDW was significantly associated with larger tumor size, positive lymph node metastases, and advanced stages; moreover, they found that the patients with an elevated RDW level had significantly lower overall survival rates. In our study elevated levels of RDW did not correlate with tumor size or metastasis or advanced stage.

In a study of the relationship between RDW and gastric cancer (GC), Erdem et al. (20) studied 68 GC patients and age-and-gender matched healthy controls. They found out that RDW was significantly elevated in GC patients when compared to healthy subjects. However, there was no statistically significant association between RDW and stage, histopathological subgroups and metastasis stage at the time of diagnosis. This finding is in line with our investigation because

we found no relationship between an elevated level of RDW and the tumor size or lymph node metastasis, but only with the grade.

Koma et al. (21) studied the relationship of lung cancer and RDW. They divided the patients as high and low RDW groups with a threshold of 15%. They revealed that RDW values were positively associated with clinical cancer stage: patients with higher RDW had more advanced disease. This may have occurred because malignancy can cause chronic inflammation and malnutrition. As a consequence, they found out that patients with higher RDW values showed poorer prognosis than the patients with lower values in the same stage disease. Although, we did not find that RDW was related to the stage of the cancer there was significant difference in RDW levels between healthy control patients and pancreatic cancer patients.

In a study of comparing 49 patients (n:35) with breast cancer and fibroadenomas (n:14), it was found that RDW was significantly higher in patients with breast cancer, when compared with those with fibroadenomas, and in the breast cancer group, RDW elevation was significantly correlated with bigger primary tumors, higher number of infiltrated axillary lymph nodes and HER2 over-expression, while it was inversely associated with the tumor grade (12). Our study also reports the inverse relationship of RDW to the grade.

In a study which aimed to discriminate obstructive jaundice from malignant obstructive cases (101 malignant vs 93 benign cases) according to the ROC curve analysis they found that a RDW level of 14.8% was the best cut-off value for predicting a malignant biliary stricture, with a sensitivity of 72% and a specificity of 69%. RDW levels were elevated (>14.8%) in 31.6% of benign cases and 68.4% of malignancies (13). In the present study with a cut-off level of 14%, the positive predictivity of RDW about malignancy was 70.73% with a negative predictive rate of 89.95% for an age-and-gender matched, randomly selected group of patients.

What lies behind the inflammation and elevation of RDW are yet far away from full elucidation; however, there are some clues. RDW has been shown to have a strong, graded association with CRP and ESR, independent of numerous confounding factors in patients with cardiovascular diseases (22). In cancer

patients, cytokines such as interleukin-6, tumor necrosis factor α , and CRP has been shown to play a key role in inducing chronic inflammation (23).

The small sample size is the major limitation of the study: however, the age-and-gender matched control group enhances the productivity of the statistical analysis. Another limitation is that RDW is not a parameter that simply reflects malignancy; it more likely reflects an inflammatory state of the body. Thus the elevated RDW must be evaluated in this context as a part of differential diagnosis of a possible malignancy. It may be argued that hemoglobin levels may effect the RDW levels but in the multiple parameter analysis RDW stands out to be solely related the grade of the tumor.

The hypothesis that an elevated RDW may be an indicator of malignancy must be studied on larger series and multiple times enough to make a meta-analysis about the possible employment of this parameter as a tumor related marker.

Conclusion

The elevated levels of RDW in a patient with a pancreatic mass may be a possible sign of malignancy, and must be carefully evaluated. However, elevated levels of RDW in a pancreatic cancer patient are not related to the stage of the disease but the grade of the disease.

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