CAN COMPLETE BLOOD CELL COUNT PARAMETERS PREDICT DEEP VEIN THROMBOSIS?

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SUMMARY – The aim of this study was to evaluate complete blood cell count parameters including red blood cell indices, white blood cell subtypes, and platelet indices for predicting deep vein thrombosis (DVT). A total of 71 (44 male and 27 female) patients with acute femoral and popliteal DVT diagnosed by doppler ultrasonography during a period of seven years (2011-2017) were included in the study. By matching age and gender, 142 (88 male and 54 female) subjects diagnosed with venous insufficiency in the same time interval were assigned as control group. Data were obtained by reviewing hospital records of the study participants, including clinical and demographic characteristics and complete blood cell parameters. Frequencies of hypertension, diabetes mellitus, chronic obstructive pulmonary disease, chronic renal failure, and coronary arterial disease were higher in DVT group as compared to non-DVT group (p<0.05). Hemoglobin and lymphocyte values were lower, and red blood cell distribution width, neutrophil, neutrophil to lymphocyte ratio, and platelet to lymphocyte ratio higher in DVT group as compared with non-DVT group (p<0.05). There was no significant between-group difference in terms of mean corpuscular volume, platelet, mean platelet volume, mean platelet volume to platelet ratio, and platelet distribution width (p>0.05). Hypertension, hemoglobin, neutrophil to lymphocyte ratio, and platelet to lymphocyte ratio were independent risk factors for DVT. We found that hypertension, anemia, neutrophil to lymphocyte ratio, and platelet to lymphocyte ratio were independent risk factors for DVT. In particular, neutrophil to lymphocyte ratio and hemoglobin may be used as novel, inexpensive, and reliable diagnostic tools for DVT.

Key words: Hypertension; Anemia; Platelet to lymphocyte ratio; Neutrophil to lymphocyte ratio; Deep venous thrombosis

Introduction

Deep vein thrombosis (DVT) is a cardiovascular disease that occurs when a blood clot forms in deep veins, usually in legs1. DVT has an incidence of approximately 1.6:1000 per year2. There are three main risk factors for DVT, i.e. hypercoagulability, venous stasis, and vascular injury1. Fine-tuning between procoagulant and anticoagulant factors is indispensable to prevent clotting of blood in vessels1. Venous stasis associated with prolonged seated immobility, such as long-distance travels, computer usage, or medical immobility including bed-rest and wheelchair usage is another important risk factor for DVT4,5. A variety of vascular injuries including extremity trauma related to repetitive motion or high-speed collisions are among causes of DVT6,7.

Recently, complete blood cell count (CBC) parameters as inexpensive and practical laboratory tests have become an interesting research area because of their potential benefits with regard to screening, prediction, classification, prognosis, and treatment monitoring of cardiovascular diseases8-11.
A great number of patients with DVT can develop post-thrombotic syndrome and pulmonary embolism that may cause major health and economic problems, particularly if no early treatment is achieved. For this reason, early diagnosis and treatment is essential to prevent the complications of DVT, and in this way to minimize morbidity and mortality and reduce health care costs.

The aim of this study was to evaluate CBC parameters including red blood cell (RBC) indices, white blood cell (WBC) subtypes, and platelet indices for predicting DVT.

Patients and Methods

This study was approved by the Ethics Committee of our Faculty of Medicine. A total of 71 (44 male and 27 female) patients with acute femoral and popliteal DVT diagnosed by doppler ultrasonography during a period of seven years (2011-2017) were included in the study. The patients were aged 20-89 years. Exclusion criteria were as follows: active cancer, surgical operation within two months, acute trauma, immobility, autoimmune diseases, and pregnancy. By matching age and gender, 142 (88 male and 54 female) subjects diagnosed with venous insufficiency in the same time interval were assigned as control group. Data were obtained by reviewing hospital records of the participants, including clinical and demographic characteristics and CBC parameters (hemoglobin levels, hematocrit, mean corpuscular volume (MCV), RBC distribution width (RDW), WBC counts, platelet count, mean platelet volume (MPV), and platelet distribution width (PDW)) measured on an ABX Pentra DX 120 (HORIBA, France) hematology analyzer. Hematologic values in the records were assayed from venous

| Table 1. Demographic and laboratory data of patients with and without DVT |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                            | DVT (+) (n=71)              | DVT (-) (n=142)             | p value                     |
| Age (yrs)                  | 55.14±17.42                 | 54.19±15.64                 | 0.0698                      |
| Gender:                    |                             |                             |                             |
| female, n (%)              | 27 (38.0)                   | 54 (38.0)                   | 1.000                       |
| male, n (%)                | 44 (62.0)                   | 88 (62.0)                   | 1.000                       |
| Hypertension, n (%)        | 21 (29.6)                   | 5 (3.5)                     | 0.000                       |
| Diabetes mellitus, n (%)   | 7 (9.9)                     | 1 (0.7)                     | 0.001                       |
| COPD, n (%)                | 8 (11.3)                    | 1 (0.7)                     | 0.000                       |
| SVD, n (%)                 | 2 (2.8)                     | 0 (0.0)                     | 0.133                       |
| CRF, n (%)                 | 6 (8.5)                     | 0 (0.0)                     | 0.000                       |
| CAD, n (%)                 | 4 (5.6)                     | 1 (0.7)                     | 0.025                       |
| Hb (g/dL)                  | 12.89±2.13                  | 14.80±1.34                  | 0.000                       |
| MCV(fL)                    | 83.5±7.81                   | 85.60±5.94                  | 0.087                       |
| RDW (%)                    | 14.00±4.60                  | 11.77±5.00                  | 0.006                       |
| Neutrophil (10^9/L)        | 5.72±2.31                   | 3.88±1.09                   | 0.000                       |
| Lymphocyte (10^9/L)        | 1.71±0.59                   | 1.71±0.59                   | 0.000                       |
| NLR                         | 3.99±3.24                   | 1.92±0.73                   | 0.000                       |
| Plt (10^9/L)               | 240.71±9.07                 | 243.08±69.60                | 0.850                       |
| PLR median (%25-75 percentile) | 133.42(98.33-187.53)     | 110.19(89.80-144.00)    | 0.010                       |
| MPV (fL)                   | 9.78±1.77                   | 9.79±1.43                   | 0.952                       |
| MPV/platelet (fL)          | 0.05±0.02                   | 0.04±0.01                   | 0.313                       |
| PDW (fL)                   | 12.70±2.88                  | 13.19±2.69                  | 0.295                       |

DVT = deep vein thrombosis; DM = diabetes mellitus; HT = hypertension; COPD = chronic obstructive pulmonary disease; SVD = cerebrovascular disease; CRF = chronic renal failure; CAD = coronary artery disease; Hb = hemoglobin; MCV = mean corpuscular volume; RDW = red cell distribution width; NLR = neutrophil to lymphocyte ratio; Plt = platelet; PLR = platelet to lymphocyte ratio; MPV = mean platelet volume; PDW = platelet distribution width
blood samples taken simultaneously with the diagnosis of acute DVT. In addition, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and MPV-to-platelet ratio were calculated.

The research procedures were performed according to the regulations of the institutional Ethics Committee and the Code of Ethics of the World Medical Association (Declaration of Helsinki).

**Statistical analyses**

Data were analyzed by using SPSS software version 20.0 (IBM, USA). In descriptive statistics, continuous variables were expressed as mean ± standard deviation (SD) and median (0.25-0.75 percentile), and categorical variables as frequency (n) and percentage (%). The participants were divided into two groups with and without DVT. The normality of distribution was assessed by Kolmogorov-Smirnov test. For numerical variables, differences between patients and controls were tested using Student’s t-test for parametric data or Mann-Whitney U test for non-parametric data. Categorical variables were analyzed using Pearson \( \chi^2 \)-test and Fisher exact test for parametric and non-parametric data, respectively. To identify the independent predictors of DVT, parameters that were significant in univariate analysis were included in multivariate logistic regression analysis.

Receiver operating characteristic (ROC) curve analysis was used to identify the optimal cut-off values and to compare predictive values for diagnosis of DVT of parameters that were significant in multivariate analysis. The significance of difference between the areas under ROC curves (AUCs) was tested by using the method of DeLong et al.\textsuperscript{14} to compare performances of predictors of DVT. The level of statistical significance was set at p<0.05.

**Results**

Demographic and laboratory data of patients with and without DVT are shown in Table 1. There was no difference between the groups in age and gender distribution (p>0.05). The frequencies of hypertension (HT), diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), chronic renal failure (CRF), and coronary arterial disease (CAD) were higher in DVT group as compared with non-DVT group (p<0.05). Hemoglobin (Hb) and lymphocyte values were lower, and RDW, neutrophil, NLR and PLR higher in DVT group as compared with non-DVT group (p<0.05). There was no significant be-
tween-group difference according to MCV, PLT, MPV, MPV:platelet ratio, and PDW (p>0.05).

Demographic and laboratory parameters (HT, DM, COPD, CRF, CAD, Hb, RDW, neutrophil count, lymphocyte count, NLR, and PLR) found to be significant in univariate analysis were then included in multivariate analysis. HT, Hb, NLR, and PLR were identified as independent risk factors for DVT. Results of univariate and multivariate analyses are depicted in Table 2.

Additionally, statistical comparisons were made for the AUCs of the parameters found to be independent predictors for DVT in multivariate analysis (Fig. 1). The AUCs for NLR, PLR, and Hb were 0.814 (95% CI: 0.744-0.884, p<0.001), 0.621 (95% CI: 0.529-0.714, p=0.028), and 0.765 (95% CI: 0.683-0.848, p<0.001), respectively. AUC values of NLR and Hb were similar to each other (p=0.3875) and higher than that of PLR (p=0.0002 and p=0.0074, respectively). The sensitivity and specificity for NLR of >1.9657 were 88.5% and 60.6%; for PLR of >170.5393 34.6% and 87.3%; and for Hb of <13.3 g/dL 57.69% and 86.62%, respectively.

Discussion

In our study, hypertension was observed in 29.6% of DVT patients and was found to be an independent risk factor for DVT. In a comprehensive study including 5063 patients with DVT, Sun et al.15 report that 55.56% of patients had hypertension. Similarly, Li et al.16 and Guo et al.17 found the frequency of hypertension in patients with DVT to be 51.9% (27/52) and 50.8% (31/61), respectively. However, there are a few studies that declare that there is no significant correlation of hypertension with DVT18,19. Also, in the study by Ren et al.20, hypertension was not a risk factor for DVT relapse. It is well known that hypertension may lead to vascular endothelium dysfunction and hypercoagulable state, and is one of the major risk factors for cardiovascular diseases21-23. Our results agree with the majority of studies on the association between DVT and hypertension, and may be interpreted as showing that hypertension made these individuals more susceptible to coagulation.

We found that anemia was an independent risk factor for DVT, which is compatible with previous studies suggesting that anemia is associated with DVT and other cardiovascular disorders24,25. This finding may be explained by two scenarios, i.e. underlying common etiologic causes related to both anemia and DVT, and adverse effects related to anemia, such as endothelial dysfunction, hypercoagulable state, and blood stasis24. There was a limitation to our study because anemia was not classified. Another study may be designed to determine association between DVT and factors leading to anemia.

It has been demonstrated that there is close association between inflammation and thrombosis26-28. Inflammation shows thrombotic effect by stimulating procoagulant factors and platelet reactivity and by inhibiting anticoagulants and fibrinolytic activity26,29. Extracellular chromatin originating from neutrophils has been reported to be prothrombotic30. These findings may explain why we found NLR as an independent risk factor for DVT. In a previous study, it was suggested that NLR may be useful in determining the extent of venous thromboembolism31. However, Roumen-Klappe et al.32 asserted that increased inflammatory markers in DVT might be a result rather than a cause of the disease.

It has been established that PLR is associated with arterial thrombosis and various cancers; however, its role in DVT has not been fully elucidated33,34. In our study, PLR was higher in patients with DVT compared to controls. Increased PLR, high platelet counts relative to lymphocyte counts, may indicate elevated predisposition to clot formation35. Recently, Mouabbi et al.35 have put forward that PLR is superior to D-dimer, increasing classically in clot situations, in terms of predicting the absence or presence of DVT. In the study by Ming et al.9, although it was found that PLR had diagnostic value in unprovoked DVT, it was not an independent risk factor for DVT. In another study, increased PLR was not associated with the risk of venous thromboembolism or cerebral vein thrombosis, but it was associated with thrombophilia abnormalities in cerebral vein thrombosis36. However, in our study, since we excluded patients with thrombophilia abnormalities, we may say that elevated PLR could have a predictive value in DVT independent of thrombophilia abnormalities.

In conclusion, we found that HT, anemia, NLR, and PLR were independent risk factors for DVT. Especially NLR and Hb may be used as novel, inexpensive, and reliable diagnostic tools for DVT. Nonetheless, these findings must be supported by further studies.
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Sažetak

MOGU LI PARAMETRI KOMPLETNE KRVNE SLIKE PREVIDJETI DUBOKU VENSKU TROMBOZU?

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Cilj ovoga istraživanja bio je procijeniti parametre kompletne krvne slike (KKS) uključujući eritrocitne indekse, podvrste leukocita i trombocitne pokazatelje za predviđanje duboke venske tromboze (DVT). U istraživanje su bila uključena 44 bolesnika i 27 bolesnica, ukupno njih 71, s akutnom femoralnom i poplitealnom DVT dijagnosticiranom Dopplerovom ultrasonografijom tijekom sedam godina (2011.-2017.) i 142 ispitanika (88 muškaraca i 54 žena) izjednačenih po dobi i spolu te s dijagnozom venske insuficijencije postavljenom u istom razdoblju kao kontrolna skupina. Potrebni podaci za sve ispitanike dobiveni su iz njihovih bolničkih zapisa, uključujući kliničke i demografske karakteristike i parametre KKS. Učestalost hipertenzije, šećerne bolesti, kronične opstruktivne plućne bolesti, kroničnog bubrežnog zatajenja i koronarne arterijske bolesti bila je viša u skupini s DVT u usporedbi sa skupinom bez DVT (p<0,05). vrijednosti hemoglobina i limfocita bile su niže, a širina distribucije eritrocita, neutrofil, omjer neutrofila i limfocita te omjer trombocita i limfocita bili su viši u skupini s DVT u usporedbi sa skupinom bez DVT (p<0,05). Nije bilo značajne razlike među skupinama u vrijednostima srednjeg korpuskularnog volumena, trombocita, srednjeg volumena trombocita, omjera srednjeg volumena trombocita i širine distribucije trombocita (p>0,05). Hipertenzija, hemoglobin, omjer neutrofila i limfocita te omjer trombocita i limfocita pokazali su se kao neovisni čimbenici rizika za DVT. Utvrdili smo da su hipertenzija, anemija, omjer neutrofila i limfocita te omjer trombocita i limfocita neovisni čimbenici rizika za DVT. Naročito bi se omjer neutrofila i limfocita te hemoglobin mogli rabiti kao novi, jeftini i pouzdani dijagnostički alati za DVT.

Ključne riječi: Hipertenzija; Anemija; Omjer trombocita i limfocita; Omjer neutrofila i limfocita; Duboka venska tromboza