Original Research Article

Evaluation of cell population data as potential markers of cobalamin and folate deficiency in populations at risk with regard to renal function

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A B S T R A C T

The purpose of the present study was to investigate neutrophil and monocyte cell population data as novel markers of low cobalamin/folate concentrations and influence of renal function on their usefulness. The study included 284 patients older than 60 years or with dyspepsia symptoms with mean corpuscular volume 80–100 fL and C-reactive protein $\leq$ 50 mg/L. Subjects were divided according to renal function and further classified based on cobalamin and folate levels. Neutrophil and monocyte volume (NeV, MoV), conductivity (NeC, MoC), light scatter (NeS, MoS) and standard deviations (NeV-SD, MoV-SD, NeC-SD, MoC-SD, NeS-SD, MoS-SD), obtained by Coulter LH750® Hematology Analyzer (Beckman Coulter, USA), were evaluated along with white blood cell count, hemoglobin, hematocrit, red cell distribution width and homocysteine relative to renal function and cobalamin/folate status. Neutrophil conductivity standard deviation (NeC-SD) had the largest magnitude of the difference between patients with low and normal vitamin levels, was the strongest predictor of low cobalamin/folate concentrations and had the largest area under the curve in detection of vitamin deficiency. Patients with different renal function status and the same cobalamin/folate status did not differ in NeC-SD. In this selected group of patients, NeC-SD was marker of low cobalamin and folate levels regardless of the renal function.

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Introduction

Cobalamin and folate deficiency causes megaloblastic anemia. Slight macrocytosis is often the earliest sign (Babior, 2005). Nuclear hypersegmentation of neutrophils is considered as an early sign in nutritional megaloblastic anemias (Babior, 2005). However, these hematological manifestations have disadvantages. Macrocytosis can be masked by coexisting disorders: iron deficiency, infection, anemia of chronic disease (Babior, 2005). Neutrophil hypersegmentation is not sensitive in mild cobalamin deficiency when changes in biochemical markers already exist (Carmel et al., 1996). Also, neutrophil nuclear segmentation is evaluated on peripheral blood smear, which is the time consuming analysis and requires experienced laboratory personal.

The new generations of cell counters automatically generate data related to leukocyte morphology. Hematology analyzers of Beckman Coulter® produce cell population data or CPD using VCS technology. This technology combines three different methods of measurement: direct current impedance for cell volume (V), radio frequency opacity for conductivity (C), which is referred to cytoplasmic/nuclear ratio, and laser light scatter (S) for cytoplasmic granularity and nuclear structure (Xu, 2015). As a result of these measurements, users obtain the mean values of volume, conductivity, light scatter and their standard deviations for leukocyte subpopulations (Krause, 1990; Xu, 2015). The cell population data are analogous to information provided by microscopic evaluation of peripheral blood smear (Xu, 2015).

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Among the groups at risk to be cobalamin deficient are older people and patients with renal or gastrointestinal diseases (Herrmann and Obeid, 2008). These groups can also overlap. Evaluation of cobalamin and folate status in patients with reduced renal function is important as a part of anemia investigation (Tsagalis, 2011) and because of the influence of chronic kidney disease (CKD) on the cobalamin and folate metabolic pathways (Bukhari et al., 2011; Carmel et al., 2001; Obeid et al., 2005). Paradoxically, high cobalamin levels can be found in the presence of functional cobalamin deficiency in patients with renal failure (Andrès et al., 2013). The high cobalamin level is defined as vitamin B_{12} concentration >701 pmol/L (950 pg/mL) (Andrès et al., 2013; Chiche et al., 2008). In the experimental animal model of CKD, Bukhari et al. (2011) demonstrated the association between CKD and down-regulation in the expression of folate transporters. Serum folate levels can be normal while cellular uptake is decreased (Bukhari et al., 2011).

The present study had two aims. First, we investigated neutrophil and monocyte CPD as potential novel hematological markers of cobalamin and folate deficiency in patients older than 60 years or with dyspepsia symptoms as groups at risk of cobalamin deficiency, with normal mean corpuscular volume (MCV). As a second aim, we evaluated the influence of renal function on the usability of CPD in the assessment of cobalamin/folate status, considering that mechanisms of renal insufficiency include activated neutrophils and monocytes (Heinzelmann et al., 1999; Yu, 2003) and can affect cobalamin and folate metabolic pathways (Bukhari et al., 2011; Carmel et al., 2001; Obeid et al., 2005).

Material and methods

Participants

The study included outpatients who were referred for biochemical testing and complete blood count (CBC) to laboratory in the Clinical Centre of Serbia. Collecting of samples was carried out during the period January 2015 – January 2016. Among 638 patients, we selected 284 subjects according to the following criteria: (1) patients did not have a diagnosis of cobalamin and folate deficiency at the time of blood sampling; (2) patients belonged to risk groups for cobalamin deficiency (age > 60 years or presence dyspepsia symptoms) and (3) mean corpuscular volume (MCV) was 80–100 fL. Exclusion criteria were values of serum cobalamin and folate higher than the upper limit of reference range (vitamin B_{12} > 675 pmol/L, folate > 45.1 nmol/L), C-reactive protein (CRP) > 50 mg/L and the use of multivitamin supplements containing cobalamin and folate during 4 weeks before blood sampling. Information on patient diseases and the use of medications were not known. Also, we selected 48 healthy subjects, 30 younger and 18 older than 60 years, in order to calculate reference intervals for novel hematological parameters. These subjects had hematological (white blood cell count, hemoglobin, hematocrit, MCV, red cell distribution width) and biochemical parameters (vitamin B_{12}, folate, homocysteine, methylmalonic acid, CRP, creatinine) within the reference range. The study was planned in accordance with standards of Helsinki Declaration and the study protocol was approved by institutional review committee of the Clinical Centre of Serbia. All patients gave informed consent before their enrolment.

Laboratory analysis

Analysis of hematological parameters along with neutrophil and monocyte CPD was performed in whole blood anticoagulated with EDTA within 4 h after collection, using Coulter® LH750 Hematology Analyzer (Beckman Coulter, USA). Neutrophil and monocyte CPD included the mean value of volume: NeV, MoV; conductivity: NeC, MoC; light scatter: NeS, MoS and their standard deviations: NeV-SD, MoV-SD, NeC-SD, MoC-SD, NeS-SD and MoS-SD.

After biochemical testing, the remaining amounts of serum samples were separated and stored at −70 °C until further analysis of vitamin B_{12}, folate, homocysteine (Hcy), creatinine and CRP. Concentrations of vitamin B_{12} and folate were determined on Access Immunoassay System® (Beckman Coulter, USA). Creatinine

Table 1
Demographic, biochemical and hematological characteristics of study group.

Parameters	eGFR < 60 mL/min per 1.73 m²		eGFR ≥ 60 mL/min per 1.73 m²	
	Group 1 (N = 62)	Group 2 (N = 33)	Group 1 (N = 105)	Group 2 (N = 84)
Male/Female	40/22	20/13	38/67	29/55
Age (years)	68	67	52	57
	(58–76)	(59–75)	(37–63)	(36–66)
WBC (10⁹/L)	6.9	6.9	6.1	6.5
	(6.0–8.2)	(6.3–9.0)	(5.1–7.3)	(5.5–7.8)
Hb (g/L)	120	121	128	120
	(115–129)	(113–130)	(119–139)	(114–134)
MCV (fL)	92	90	93	90
	(88–95)	(86–93)	(89–96)	(87–93)
Hct (%)	36	37	38	37
	(34–38)	(34–39)	(36–41)	(34–40)
RDW (%)	14.4	14.4	13.7	14.2
	(13.6–14.8)	(13.5–16.0)	(13.2–14.5)	(13.6–15.0)
B12 (pmol/L)	245	129	228	126
	(183–297)	(103–147)	(184–306)	(104–146)
Folate (nmol/L)	16.9	14.1	16.2	13.4
	(13.2–22.8)	(8.5–19.4)	(13.6–21.9)	(8.5–20.1)
Hcy (μmol/L)	20.6	25.1	11.7	12.8
	(16.2–25.2)	(19.9–33.0)	(9.5–14.7)	(10.5–16.7)
Cr (μmol/L)	144	131	67	68
	(125–179)	(107–195)	(61–78)	(60–78)
CRP (mg/L)	1.9	2.4	1.5	2.1
	(0.9–4.7)	(1.0–6.3)	(0.9–3.0)	(0.9–5.3)

eGFR: estimated glomerular filtration rate, WBC: white blood cell count, Hb: hemoglobin, MCV: mean corpuscular volume, Hct: hematocrit, RDW: red cell distribution width, B_{12}: vitamin B_{12}, Cr: creatinine, Hcy: homocysteine, CRP: C-reactive protein