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Role of NEUT-X & NEUT-Y in picking up megaloblastic anaemia on peripheral blood & in differentiating from other macrocytic anaemia

Sir,

Macrocytic anaemia refers to an anaemic state, in which red blood cells (RBC) are larger than normal, recognized by automated RBC indices and confirmed on peripheral blood smear. Macrocytosis is relatively common with a prevalence ranging from 1.7 to 3.6 per cent[1]. The causes are broadly categorized into megaloblastic and non-megaloblastic. The megaloblastic causes comprise nutritional deficiencies such as vitamin B_{12}/folate deficiency. The prevalence of subnormal vitamin B_{12} concentration in elderly varies from 3 to 40.5 per cent depending on the cut-off used for defining deficiency of cobalamin level in serum[2]. The non-megaloblastic causes commonly include drugs, alcoholism, liver diseases and hypothyroidism along with primary bone marrow disorders such as myelodysplasia, leukaemia, multiple myeloma and aplastic anaemia[1,3]. The gold standard for differentiating between these two major categories of macrocytic anaemia is based on a comprehensive approach with clinical history, high mean corpuscular volume (MCV) and/or red cell distribution width (RDW), vitamin B_{12} levels, peripheral blood smear and bone marrow examination.

The MCV represents the mean of the distribution curve and is useful in recognizing macrocytosis. Macrocytosis is considered when MCV is >100 fl (normal range 80-100 fl); the reference range, however, can vary between laboratories and also depends on the age of the patient[1,3]. As MCV is the mean which is calculated, it is insensitive to the presence of a small number of macrocytes. Further, macrocytosis can be obscured by concomitant disorders that can cause microcytosis, resulting in masked megaloblastic anaemia (MA), for example, iron deficiency, hereditary elliptocytosis, alpha and beta thalassemia, haemoglobin H disease or fragmentation[4]. Blood transfusion can also cause spurious masked macrocytosis. In such conditions, it is difficult to pick up MA from MCV alone as the MCV may remain low or normal (as it is mean value). In such cases, RDW will be high due to the variation in the size of RBCs which is again not a very sensitive parameter but can be used as an adjunct for further investigations.

In MA, the peripheral blood smear shows cytopenia with macroovalocytes and hypersegmented neutrophils (>5 lobes in 5% neutrophils, six lobes nuclei or lobe average of >3.5)[5]. When the MCV is low due to the presence of concomitant microcytic conditions, structural changes in neutrophil may be the only morphologic clue to a megaloblastic aetiology of anaemia[4]. The macroovalocytes seen in a typical case of MA are a direct result of ineffective haematopoiesis due to defective DNA synthesis[1].

The role of NEUT-X and NEUT-Y is well known in myelodysplastic syndrome cases[6]. In our previous study[7], 10 samples were run twice in 24 h to determine the stability. The samples were stored at 2-8°C. The mean values of NEUT-X and NEUT-Y on the 1st and 2nd runs were comparable, suggesting a reasonable stability of both NEUT-X and NEUT-Y over a 24 h period[7]. This study was undertaken to evaluate the role of NEUT-X and NEUT-Y in detecting MA on peripheral blood and also see whether other forms of macrocytic anaemia can be differentiated from MA.

In the present study consecutive patients fulfilling inclusion criteria were retrospectively selected over a period of one year (September 2013 to July 2014) in a multimodality tertiary care hospital (Medanta- The Medicity hospital) in Gurugram, India. The study comprised three groups: controls, MA and non-megaloblastic macrocytic anaemia (MacA). Control group included 64 randomly selected healthy

individuals, which came for regular health check-up with no co-morbidities/complaints. These were compared with 44 patients with MA and 40 with MacA. The patients were diagnosed by following multiparametric approach comprising clinical history, peripheral blood examination including counts and smear, biochemical markers including vitamin B_{12}/folate levels, liver function tests, renal function tests, thyroid function tests and serum iron studies. Where required bone marrow examination for diagnosis of megaloblastic and other macrocytic anaemia cases was also done. The inclusion criteria for MA patients were low vitamin B_{12}/folate levels, anaemia (men <13 g/dl and women <12 g/dl) with or without high MCV and confirmed on bone marrow examination. The inclusion criteria for other macrocytic anaemia cases were normal or high vitamin B_{12}/folate levels, anaemia with MCV >100 fl and proven cause of macrocytosis apart from nutritional deficiency either biochemically or on bone marrow examination. The exclusion criteria were newborns and pregnant women, and those with reticulocytosis, spurious macrocytosis and macrocytosis without anaemia. The study protocol was approved by the institutional ethics committee and written informed consent was obtained from all participants.

NEUT-X and NEUT-Y counts were performed on Sysmex XE–2100, Sysmex, Kobe, Japan. Biochemical markers were done on Vitros 1500 (Johnson & Johnson, USA) Vitamin B12 level was done by competitive assay methodology. The blood samples (3 ml in EDTA) were run within two hours of collection avoiding delayed processing and storage. Three levels of internal quality controls provided by Sysmex, Japan, were run thrice daily as part of internal quality in XE–2100.

Statistical analysis was performed using one-way analysis of variance followed by Dunnett's test for multiple comparisons versus control group. All statistical analyses were performed using SPSS statistical version 16.0 software package (SPSS Inc., Chicago, IL, USA).

The study groups comprised 64 individuals of control group, 44 patients with MA and 40 with MacA. The 40 patients of other macrocytic anaemia included 17 with alcoholism and liver disease, eight with MacA secondary to drug intake, two of chronic renal disease, three of hypothyroidism, five of aplastic/hypoplastic anaemia and five patients with multiple myeloma. The mean age of control, MA and MacA groups were 52.44 ±13.91, 46.21 ±18.13 and 50.28 ±16.17 yr, respectively, indicating no significant difference in the age among the study groups (Table). The mean haemoglobin value in control group was 13.3 g/dl, whereas it was significantly (P<0.001) lower in MA and MacA groups, respectively (P<0.001) (Table). There was no significant difference between MA and MacA groups, indicating that haemoglobin alone cannot be used to differentiate these two categories.

The mean MCV in MA and MacA groups was 99.10 and 107.0 fl, respectively, significantly (P<0.001) higher than the control group (86.2 fl). MCV of MacA group was significantly higher than MA (P<0.01). The MCV in MA group ranged from 62.9 to 131.4 fl, as 23 of the 44 MA patients (52.3%) had concomitant iron deficiency anaemia, thus lowering the MCV values and giving a wide range. When only NEUT-X was used as the screening parameter, 37 of the 44 MA cases (84.1%) were detected which was significant when compared to the 21 of the 44 MA cases (47.7%) where MCV (>100 fl) was used alone as a screening parameter (P<0.05). The mean value of RDW (CV%) in control group was 14.06 which was significantly (P<0.001) lower than MA and MacA groups (20.84 and 19.82). However, no significant difference was noted in the red cell distribution width (RDW) value between MA and MacA groups. RDW has been suggested as an additional marker with macrocytes in peripheral smear, in picking cases with macrocytosis[1]. However, in the present study, RDW was useful only when compared to the control group and not with the other MacA.

NEUT-X value was significantly higher in the MA group (1445.5; P<0.001) and MacA group (1354.0; P<0.05) compared to the control group (Table), similar to our previous study[7]. NEUT-Y value was significantly higher in the MA group (422.18; P<0.001) when compared to the control group, which was also comparable with the previous study[7]. On comparison between MA and MacA groups, the high values of both parameters (NEUT-X and NEUT-Y) in MA group were significant (P<0.001). Using linear regression formula, poor correlation was noted between MCV and NEUT-X in control, MA and MacA groups and no correlation between MCV and NEUT-Y in the above groups, indicating that NEUT-X and NEUT-Y parameters can be used as independent markers in the pickup of MA cases.

In conclusion, our study shows that higher values of NEUT-X and NEUT-Y in MA patients, in spite of low or normal MCV, high RDW, may be used to not only reveal the masked cases of MA with concomitant

Table. Significance of age, haemoglobin level, mean corpuscular volume, red cell distribution width, NEUT-X and NEUT-Y in control (n=64), megaloblastic anaemia (MA) (n=44) and macrocytic anaemia (MAC) (n=40) groups

Groups	Mean	SD	SEM	CL		Maximum	Minimum
				Upper limit (mean + 1.96 SD)	Lower limit (mean - 1.96 SD)		
Age (yr)							
Control	52.44	13.91	1.74	79.70	25.18	80	25
MA	46.21	18.13	2.73	81.74	10.68	75	2
MAC	50.28	16.17	2.51	81.97	18.59	76	16
Haemoglobin (g/dl)							
Control	13.3	1.60	0.198	16.44	10.16	17.4	11.8
MA	7.14***	1.718	0.259	10.51	3.77	10.4	3.9
MAC	7.77***	1.788	0.283	11.27	4.26	10.6	3.2
MCV (fl)							
Control	86.17	4.53	0.571	95.05	77.29	99.2	80.0
MA	99.10***	15.19	2.29	128.87	69.32	131.4	62.9
MAC	107.0***,††	5.51	0.871	117.80	96.20	117.8	98.2
RDW (CV %)							
Control	14.06	1.28	0.16	16.57	11.55	18.4	12.2
MA	20.84***	5.06	0.76	30.76	10.92	33.5	11.9
MAC	19.82***	4.85	0.77	29.33	10.31	28.8	12.9
NEUT-X							
Control	1335.1	28.3	3.57	1390.6	1279.6	1416	1277
MA	1445.5***	61.14	9.22	1565.3	1325.7	1539	1309
MAC	1354.0*,†††	43.37	6.86	1439.0	1269.0	1401	1190
NEUT-Y							
Control	390.3	16.6	2.10	422.8	357.8	440	352
MA	422.18*,†††	40.96	6.17	502.46	341.90	550	338
MAC	391.43†††	40.02	6.33	469.87	312.99	498	306

P^{*}<0.05 and ***<0.001 compared to control group and $P^{††}$<0.01 and †††<0.001 compared to MA group. MCV, mean corpuscular volume; RDW, red cell distribution width; SEM, standard error of mean; CL, confidence limit

iron deficiency but also separate them from the broader macrocytic anaemia group. Peripheral smear remains diagnostic; however, making smear for all the suspicious cases is a cumbersome and tedious procedure. Thus, these parameters emerge as more reliable and stable parameters in the modern laboratories, which in future may replace the older screening tools. However, prospective studies should be done to evaluate sensitivity, specificity and behaviour of these parameters (whether these return to normal levels) in the early phases of the disease or following treatment of such cases.

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***Conflicts of Interest*:** None.

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References

1. Aslinia F, Mazza JJ, Yale SH. Megaloblastic anemia and other causes of macrocytosis. *Clin Med Res* 2006; *4* : 236-41.
2. Shobha V, Tarey SD, Singh RG, Shetty P, Unni US, Srinivasan K, *et al*. Vitamin B_{12} deficiency & levels of

metabolites in an apparently normal urban South Indian elderly population. *Indian J Med Res* 2011; *134* : 432-9.

3. Kaferle J, Strzoda CE. Evaluation of macrocytosis. *Am Fam Physician* 2009; *79* : 203-8.
4. Gulley ML, Bentley SA, Ross DW. Neutrophil myeloperoxidase measurement uncovers masked megaloblastic anemia. *Blood* 1990; *76* : 1004-7.
5. Wintrobe M, Lee GR, Boggs DR, Bithell TC, Foerster J, Athens JW, *et al*. *Clinical Hematology*, 8th ed. Philadelphia, PA: Lea & Febiger; 1981. p. 573.
6. Le Roux G, Vlad A, Eclache V, Malanquin C, Collon JF, Gantier M, *et al.* Routine diagnostic procedures of myelodysplastic syndromes: Value of a structural blood cell parameter (NEUT-X) determined by the Sysmex XE-2100™. *Int J Lab Hematol* 2010; *32* (6 Pt 1) : e237-43.
7. Goel S, Sachdev R, Gajendra S, Jha B, Sahni T, Dorwal P, *et al.* Picking up myelodysplastic syndromes and megaloblastic anemias on peripheral blood: Use of NEUT-X and NEUT-Y in guiding smear reviews. *Int J Lab Hematol* 2015; *37* : e48-51.