Differential Leucocyte Alkaline Phosphatase Activity (LAPA) in Chronic Myeloid Leukaemia (CML) and Myeloid Leukemoid Reaction (MLR)

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Abstract

Leucocyte Alkaline Phosphatase Activity (LAPA) in normal healthy individuals was lound to be in the range 17-110. It was markedly increased in patients with myeloid leukemoid reaction (MLR) in the range 100-220. However it was decreased in patients with chronic myeloid leukaemia (CML) in the range 0-16. Increased LAPA in MLR is indicative of enhanced digestive capability of neutrophils while decreased values in CML are suggestive of defective neutrophil function. LAPA is a diagnostic parameter in differentiating between MLR and CML and also seem to have a prognostic and predictive value for blastic transformation in CML (JPMA 34: 114, 1984).

Introduction

Polymorp honuclear leucocytes contain a large number of enzymes and some of these are important in digestion of phagocytosed material. A proportion cf neutrophil leucocytes contain the enzyme Alkaline phosphatase which is thought to be involved in the digestion process. A number of cytochemical techniques are available for the demonstration of this enzyme at light microscopy (Menten et al., 1944; Wiltshaw and Moloney, 1955; Kaplow, 1955; Ackerman, 1962; Kaplow, 1968). A scoring system was devised for leucocyte alkaline phosphatase activity (LAPA) where hundred segmented and band form neutrophilic granulocytes are rated from 0 to +4 depending on the amount and intensity of staining (Kaplow, 1968). Normal range of LAPA has been reported as 11-95 (Kaplow, 1968). Increased LAPA scores have been reported in a variety of clinical conditions, these include aplastic anaemia, agranulocytosis, Lymphoma, myeloid leukem oid reactions, chronic lymphocytic leukaemia and polycythemia vera and conversely low values have been reported in chronic myeloid leukaemia (Hayhoe and Quaglino,1958; Kaplow, 1968). In this study we report LAPA scores in differenciating myeloid leukemoid reactions from chronic myeloid leukaemia and LAPA value in predicting prognosis and course of the disease in chronic myeloid leukaemia.

Material and Methods

Slides were made with EDTA and Heparin anticoagulated and fresh blood (no anticoagulant) from 6 apparently normal healthy individuals and 16 patients with reactive neutrophilic leucocytosis. Morphology was assessed on EDTA blood slides stained with Leishmann. Leucocyte alkaline phosphatase activity was determined on fresh blood or heparanised blood films by the method of Ackerman (1962) using a leucocyte alkaline phosphatase kit (Sigma). Leucocyte alkaline phosphatase activity score was assessed by the method of Kaplow (1968). Essentially 100 segmented and band neutrophils were rated as follows: no activity, +1: upto 50% of the cytoplasm occupied by small granuoles, +2: upto 80% of the cytOplasm occupied by small granuoles, +3: 80-100% of the cytoplasm occupied by medium to large granuoles and +4: 100% of the cytoplasm occupied by medium to large

granuoles with intense and bright staining After LAPA was performed, 16 patients studied were diagnosed as Chronic myeloid leukaemia (CML) or Myeloid leukemoid reaction (MLR) on the basis of clinical and haematological features and LAPA scores. Bone marrow was done in seven of the sixteen cases studied.

Results

LAPA was determined in 6 normal healthy individuals (Table I)and the range was found to be

Table I LAPA in Normal Healthy Individuals.

Subject	LAPA	Total Count x 10^9/L	Myeloid cells % of Total Differential Segmented	Band	Metamyelo	Myelocyte
1	110	6.5	65	01	nil	nil
2	30	8.0	58	nil	nil	nil
3	22	9.5	62	01	nil	nil
4.	17	5.8	59	nil	nil	nil
5.	60	11.0	60	nil	nil	nil
6.	80	7.5	49	nil	nil	nil

17-1 10. LAPA was increased in patients with Myeloid Leukemoid reaction (Table II)

Table –II LAPA in Patients With Myeloid Leukemoid Reaction (MLR).

Subject	LAPA	Total count x 10^9/L	Myeloid cells % of Total Differential Segmented	Band	Metamyelo	Myelocyte
!	150	40.0	64	15	05	01
2.	200	31.0	65	16	02	01
3.	210	28.0	72	06	08	02
4.	131	36.0	75	10	02	02
5.	100	22.0	67	14	05	03
6.	189	29.0	70	20	nil	nil
7.	220	31.0	62	19	01	01
8.	175	28.0	60	26	nil	nil
9.	115	45.0	70	20	02	nil

with a range of l00.220 diagnosed as Chronic me values were much lower man the normals

Table –III LAPA in Patients With Chronic Myeloid Leukaemia (CML).

Subject	LAPA	Total count x 10^9/L	Myeloid cells % of Total Differential Segmented	Band	Metamyelo	Myelocyte
1	01	110.0	35	27	15	10
2.	15	70.0	40	30	10	02
3	04	56.0	30	20	10	20
4	10	220.0	38	30	10	05
5	16	47.0	35	25	10	10
6	00	170.0	20	30	20	20
7	00	95.0	20	20	20	20

Table III with a range of0-16.Patients 1,6 and 7 with low scores of LAPA at presentation went into blastic transformation of CML at 8, 11 and 12 months respectively. The rest have remained in the chronic phase of CML. Low scores of LAPA in this small series of patients are related to an early onset of blast transformation.

Discussion

Leucocyte Alkaline Phosphatase is thought to be involved in the digestion of phagocytosed material in neutrophils. The increase in LAPA observed in MLR is suggestive of enhanced digestion capacity of neutrophils to fight infection. Conversely decreased values in CML are suggestive of functional abnormalities of neutrophils. Most cases of MLR can be distinguished from CML on careful consideration of the clinical and haematological features. However when confusion is at hand LAPA is helpful in differenciating the two, specially when clinical features are not clear, the total Leucocyte count is below 100,000/cmm in CML or above 50,000/-cmm in MLR. In this study patient 1 (Table II) was diagnosed as CML on peripheral film and bone marrow, however a simultaneous sample was sent to us and after LAPA score diagnosis was changed to MLR and treated as such. Conversely pat ent 5 (Table III) presented with the diagnosis of MLR due to absence of clinical features. Simultaneous blood tests and LAPA scores at our centre suggested CML. Three weeks later the same patient presented with a count of 135,000/cmm with clinical and haeinatological features of CML. In the limited experience of 14 cases of CML (present series included), 50% of the patients presented with initial counts of less than l00,000/cmm. This is contrary to Western observations where counts cf greater than l00,000/cmm are usually encountered. In this regard LAPA has been of diagnostic significance in differenciating between MLR and CML in this series. Secondly low LAPA in CML at diagnosis predict an early onset of blastic transformation in this series. This needs to be evaluated further with larger series of patients to give LAPA a predictor, prognostic value along with its diagnostic significance in CML.

Acknowledgements

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References

1. Ackerman, G.A. (1962) Substituted napthol AS phosphate derivatives for localisation of leucocyte alkaline phosphatase activity. Lab. Invest., 11:563.
2. Hayloc, F.G. and Quaglins, G.D. (1958) Cytochemical demonstration and measurement of leucocyte alkaline phosphatase activity in normal and pathological states by a modified azo-dye coupling technique. Br. J. Haematol., 4:375.
3. Kaplow, L.S. (1955) A histochemical procedure for localising and evaluating leucocyte alkaline phosphatase activity in smears of blood and bone marrow. Blood, 10:1023.
4. Kaplow, L.S. (1968) Leucocyte alkaline phosphatase; cytochemistry applications and methods. Ann. N. Acad. Sci., 155:911.
5. Menten, M.L., Junge, J. and Green, M.H. (1944) Coupling histochemical Azo dye test for alkaline phosphatase in kidney. J. Biol. Chem., 153 : 471.
6. Wiltshaw, E. and Moloney, W.C. (1955) Histochemical and biochemical studies on leukocyte alkaline phosphatase activity. Blood, 10:1120.