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The Challenges of Laboratory Diagnosis of Megaloblastic Anaemia in Nigeria

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ABSTRACT

In Nigeria, most primary health care facilities are without a laboratory or poorly structured laboratories. The diagnosis is therefore made on the basis of clinical symptoms and preliminary laboratory investigations, where available. Because emphasis is not placed on complex laboratory methods for the characterization of anaemia in the course of training, laboratory personnel usually under diagnose megaloblastic anaemia. This paper reviews diagnostic methods ideal for megaloblastic anaemia.

INTRODUCTION

Megaloblastic anaemia is group of anaemia resulting from defective DNA synthesis leading to delayed maturation of the nucleus of erythroblasts as compared to the maturation of their cytoplasm. The defect in DNA synthesis is most commonly due to deficiency of vitamin B12 or folic acid, or sometimes due to abnormalities of metabolism of these substances. The defect in DNA synthesis occurs because vitamin B12 and folic acid act as coenzymes in the synthesis of DNA (Ochei and Kolhatkar, 2000).

According to Ezimah (2001), other causes of megaloblastic anaemia include: drugs e.g. vinca alkaloids, cystosine arabinoside, 6-mercaptopurine, anti-folate compounds etc., leukaemia, diGuglielmo's syndrome and congenital disorders such as orotic aciduria, congenital dyserythropoietic anaemia, juvenile pernicious anaemia, etc.

Biermer anemia, Addison's anemia, Vitamin B12 deficiency anemia, Pernicious anemia are synonyms often used to describe megaloblastic anaemia depending on the etiology. Adediran *et al.*, (2003) observed that malaria and nutritional deficiencies are the major cause of severe anaemia in Nigerian children. In a study of Pernicious Anaemia in Africans, Akinyanju and Okany (1992) observed that the incidence of megaloblastic anaemia (Pernicious) is higher among females than in males.

Megaloblastic anaemia is prevalent in Nigeria and patients are diagnosed late (Baznaye *et al.*, 2005). According to Akinyanju and Okany (1992), the reluctance to consider the diagnosis of magaloblastic anaemia is due to firmly held notions of it rarity and a penchant for empirically treating chronic anaemias with all available haematinics and blood transfusion. This has contributed to the underdiagnosis of megaloblastic anaemia in Nigeria.

Since megaloblatic anaemia results from multi-factorial nutritional and or metabolic / immunological considerations. A specific diagnosis including the laboratory identification of the etiological agent must be made to correct the disorder. This is because administration of drugs, haematinics or transfusion does not always correct anaemia. In a study of bone marrow status of anaemic pregnant women on supplemental iron and folic acid in a Nigerian community, Okafor *et al.*, (1985) observed that 35.4% had megaloblastic changes in the bone marrow.

DIAGNOSIS OF MEGALOBLASTIC ANAEMIA (THE CASE IN NIGERIA AND THE IDEAL)

In Nigeria, most primary health care facilities are without a laboratory or poorly structured laboratories. The diagnosis is therefore made on the basis of clinical symptoms and preliminary laboratory investigations, where available. Because emphasis is not placed on complex laboratory methods for the characterization of anaemia in the course of training, laboratory personnel usually under diagnose the condition.

Hoffbrand and Provan (1997), reported the following tests as those commonly performed in primary care for the diagnosis of megaloblastic anaemia:

- **Full blood count**
  - This may show low haemoglobin and increased mean cell volume (MCV) - although macrocytosis can precede the development of anaemia. Severe cases may show a pancytopaenia.
  - The reticulocyte count may be low for the degree of anaemia (1-3% only).
  - The MCV may be normal if there is associated iron deficiency.

- **The blood film** This may show macrocytic red cells, neutrophils with hypersegmented nuclei and Howell-Jolly bodies (residual fragments of the nucleus causing spherical blue-black inclusions on red blood cells seen on Wright-stained smears. Associated iron deficiency may result in the MCV being normal, in which case two types or red blood cells may be seen (a dimorphic blood film. The ferritin level should be checked if such a picture is seen.
- **Biochemistry**
    - o There may be an increase in plasma unconjugated bilirubin due to increased destruction of red-cell precursors in the marrow. Liver and thyroid function tests, and protein electrophoresis may help in the differential diagnosis of macrocytosis.
    - o Serum vitamin B12 is the most commonly used method of establishing B12 deficiency. In general, levels < 150 pg/ml reliably indicate deficiency. Neurological deficiency or anaemia is usually evident in patients with levels < 120 pg/ml. False positives (low levels in the absence of deficiency) can occur with pregnancy, folate deficiency(Mollin *et al.*, 1976), myeloma (Vlasveld , 2003) and excessive vitamin C intake.
    - o False negatives (normal levels in the presence of deficiency) may occur in true deficiency (Lechner, 2005), liver disease, lymphoma, autoimmune disease, and myeloproliferative disorders. In borderline cases or where B12 deficiency is clinically suspected, other tests must be carried out. Tissue deficiency of B12 results in raised levels of serum methylmalonic acid, and this is a useful test where false positive of negative values are suspected. Other tests include trancobalamin II-B12 content, and plasma total homocysteine (Wickramasinghe, 2006).
    - o Folic acid levels should be measured to exclude deficiency, which may co-exist with B12 deficiency. Red-cell folate is a better guide to deficiency than serum folate. B12 deficiency may result in increased serum folate levels but reduced red-cell folate levels, because of the effect on intracellular folate metabolism (Haltmayer *et al.*, 2002). Combined deficiency usually results in both reduced serum folate and vitamin B12 levels.
- **Autoantibody screen** Intrinsic factor (IF) antibodies, if present, are virtually diagnostic of pernicious anaemia. However, they are absent in 50% of patients with pernicious anaemia. Gastric parietal-cell antibodies are present in 85% of people with pernicious anaemia, but are also found in 3-10% of people who do not have pernicious anaemia.

Full blood count and blood film examination are efficiently done in most laboratories in Nigeria. The biochemical tests (serum vitamin B12 and folate estimation) are not routinely carried out due to poor facilities and poor training. Where the diagnosis can be made, it is usually done at the Secondary or Tertiary health care facilities. The estimation of folate and serum vitamin B12 should however form part of the diagnosis of megaloblatic anaemia at the primary health care level to exclude other causes of macrocytosis for prompt medical care.

Tests which may be performed in secondary care

- **The Schilling test** The purpose of this test is to differentiate patients whose B12 deficiency is due to pernicious anaemia from those who have an intestinal lesion causing malabsorption. It measures the absorption of B12 with and without intrinsic factor.
    - o The patient must not take B12 for five days prior to the test.
    - o Radioactive B12 is given orally followed in one to six hours by a parental B12 'flushing' dose (1000 mcg) to avoid liver storage of radioactive B12.
    - o The percentage of radiolabelled material in a 24 hour urine collection is then measured (normally > 9% of the dose given).
    - o Reduced urinary excretion (< 5%) in the presence of normal kidney function supports the diagnosis of decreased absorption of vitamin B12.
    - o Repeating the first test (Schilling I) using radiolabelled cobalt attached to intrinsic factor from a hog (Schilling II) will confirm if absorption is increased, thus supporting the diagnosis of pernicious anaemia.

  The Schilling test has its limitations:

    - o Radiolabelled vitamin B12 is difficult to obtain, it is complicated to perform, and test results can be difficult to interpret in (often elderly) patients with renal insufficiency (**Oh and Brown,** 2003)
    - o Because the Schilling test does not measure absorption of food-bound B12, the test will not detect defective liberation of food-bound B12 in the elderly patient (Andres *et al.*, 2004). Furthermore, the test result often does not contribute much to the ultimate management of the patient.

  If a Schilling test is felt inappropriate, in elderly patients with a low vitamin B12 level and negative intrinsic factor antibodies, response to vitamin B12 may be adequate to confirm a diagnosis of pernicious anaemia if:

    - o The person feels better in 1-2 days
    - o The reticulocyte count increases in 2-3 days, and peaks in 5-8 days
    - o The red blood cell count increases within 1 week, and normalizes in 4-8 weeks
    - o The MCV increases for 3-4 days (due to the increased reticulocyte count), then decreases, reaching the normal range in 25-78 days
    - o Haemoglobin level increases by 2-3 g/dl every 2 weeks
    - o White blood cell and platelet counts normalise in 7-10 days
- **Bone-marrow aspiration** may be necessary to narrow the differential diagnosis, especially if myelodysplasia, aplastic anaemia, myeloma, or other marrow disorders are suspected. In B12 and folate deficiency, megaloblasts and giant metamyelocytes (early granulocyte precursors) are seen.
- **Gastroscopy** is appropriate on diagnosis to confirm gastric atrophy and exclude gastric cancer and polyps(Ye and Nyren, 2003). Gastric cancer is two to three times commoner in patients with pernicious anaemia than in matched controls.

These tests are rarely performed in secondary or tertiary health care facilities in Nigeria. The challenge is therefore to include these investigations in the scheme of analysis for all suspected cases of Meglablastic anaemia.

DIFFERENTIAL DIAGNOSIS OF MEGALOBLASTIC ANAEMIA

Megaloblastic anaemia is often misdiagnosed in Nigeria when the patient has other causes of macrocytosis. Occasionally, the morphological changes in megaloblasts and other cells may be extremely bizarre; these changes have been misinterpreted as neoplasia, acute leukemia, or myelodysplasia.

According to Paul, (2007) the following pattern may be employed in the differential diagnosis of megaloblastic anaemia.

Laboratory Studies

- A CBC count, RBC indices, platelet count, differential count, reticulocyte count, and microscopic examination of the peripheral blood smear should be performed.
    - A typical patient with megaloblastic anemia presents with macrocytic anemia with thrombocytopenia and a decreased reticulocyte count. The mean cell volume can range from 100-150 fL or greater.
    - Hypersegmented neutrophils can be observed on the peripheral smear and represent an early phase of megaloblastosis in persons with nutritional megaloblastic anemias. Hypersegmented neutrophils contain 5 or more lobes, while normal neutrophils contain 3-4 lobes.
    - Macrocytes are oval and have been called macroovalocytes. In persons with severe anemia, macrocytes with nuclear remnants and erythrocytes with megaloblastic nuclei can be present in the peripheral blood. Macrocytes can be found in the peripheral blood in patients with liver disease or hemolytic anemia (because of an increase in reticulocytes) and usually do not have oval features. However, macroovalocytes are characteristic of megaloblastic anemias.
    - In general, the profoundness of megaloblastic changes is proportional to the severity of the anemia.
    - In some cases of megaloblastosis, no anemia is present despite overt neuropsychiatric disease. One cause of this disparity is the administration of folic acid to patients with cobalamin deficiency. This therapy partially corrects the anemia, but the neuropathy is not affected and progresses.
    - Macrocytosis due to cobalamin or folate deficiencies may be masked in patients with microcytic anemias because of thalassemia or iron deficiency. However, hypersegmentation of neutrophils may persist. Transfusion therapy or infections may modify the expression of megaloblastosis.
- Lactodehydrogenase and indirect bilirubin assays should be ordered, and results are expected to be high because of intramedullary destruction of megaloblastic red cell precursors. LDH fraction 1 (LDH_1) and LDH fraction 2 (LDH_2) are elevated, with LDH_1 being greater than LDH_2. The LDH level is often extremely high, and, following therapy, the fall in the LDH level is an excellent indication of response to or failure of therapy. Increased LDH and indirect bilirubin levels along with a decreased reticulocyte count suggest ineffective hemopoiesis in which intramedullary hemolysis is occurring.
- Serum iron and ferritin assays should be ordered initially and during the treatment of megaloblastic anemias. These parameters may be high. Increased iron turnover occurs in persons with untreated megaloblastosis. However, serum iron and ferritin levels may also decrease because patients respond to therapy and consume iron stores for the production of new RBCs. If iron stores are depleted, patients have an incomplete response to cobalamin or folate therapy.
- Tests for the diagnosis of cobalamin deficiency are described as follows:
    - The most important test is measuring the serum cobalamin level. In a typical clinical presentation of megaloblastic anemia, a low serum cobalamin level and a full response to cobalamin may be sufficient to establish a diagnosis. A Schilling test can be performed in patients who have been treated with cobalamin and folate. This test can be used to diagnose cobalamin deficiency and to distinguish between pernicious anemia and ileal malabsorption.
    - Serum for cobalamin levels should be drawn before transfusions or vitamin B-12 therapy. If the test cannot be performed within a reasonable time frame, serum should be frozen to preserve it for testing so that therapy can be started. Serum cobalamin levels are usually low in patients with anemia due to cobalamin deficiency. However, exceptions to this rule exist.
    - Cobalamin levels may be falsely high in patients with megaloblastosis due to nitrous oxide, TCII deficiency, inborn errors in cobalamin metabolism, and myeloproliferative disorders. On the other hand, serum cobalamin levels can be falsely low with normal tissue levels in some patients with folate or iron deficiency, vegetarians, individuals on high doses of ascorbic acid, pregnant women, and persons with transcobalamin I (TCI) deficiency.
    - Serum samples for folate levels should also be obtained and, if necessary, frozen prior to therapy in patients with possible cobalamin deficiency because patients with folate deficiency can have reduced cobalamin levels.
    - A Schilling test is a radiometric test of cobalamin absorption. The test is given in 3 parts, as follows:
        - In the first part of the test, radioactive cyanocobalamin is given orally. Unlabeled cyanocobalamin is given intramuscularly to inhibit the uptake of radioactive cobalamin by the liver. Next, the urinary secretion of radioactive cobalamin is measured to estimate whether the orally administered cobalamin has been taken up. Low secretion suggests either pernicious anemia or an abnormality in the terminal ileum that prevented the uptake of IF-cobalamin complexes.

        - The second part of the test is performed in the same manner, except that IF is given orally along with radioactive cyanocobalamin. If IF restores the uptake of ingested radioactive cyanocobalamin, the patient most likely has pernicious anemia. However, if IF does not restore uptake, then an abnormality in the terminal ileum is most likely present.
        - A third phase can be performed in which the patient is treated with antibiotics prior to administering radioactive cyanocobalamin. If antibiotics restore cobalamin absorption from the gastrointestinal tract, the patient most likely has a blind loop syndrome.
        - The main difficulty with the Schilling test is inadequate collection of urine samples in patients who are either noncompliant or in renal failure.
        - The results of the Schilling test may indicate cobalamin malabsorption in patients who have severe and long-standing folate deficiencies. This is because of the effect of severe folate deficiency on the ileal mucosa that leads to a decrease in cobalamin uptake in the terminal ileum. Treating patients with severe folate deficiency with both cobalamin and folate for a month may be advisable to restore the ileal mucosa before performing a Schilling test.
    - A protein-bound absorption test (also known as food-cobalamin absorption test) should be performed if food-cobalamin malabsorption is suggested. In this disorder, IF is present, but cobalamin bound to r-binder is not released and thus cannot bind to IF. Results of a standard Schilling test are normal in persons with this disorder. However, if the Schilling test is modified by using in vivo cyanocobalamin-radiolabeled food or in vitro cyanocobalamin-radiolabeled chicken serum or eggs instead of free radiolabeled cyanocobalamin, the Schilling test result will be abnormal. The results of the modified Schilling test can help detect the failure of the release of cobalamin bound to foods.
    - Methylmalonic aciduria is another test. Urinary excretion is a reliable index of cobalamin deficiency, provided the patient does not have renal failure.
    - Serum methylmalonic acid and homocysteine test results are elevated in more than 90% of patients with cobalamin deficiencies.
    - Antiparietal cell antibodies are rarely ordered in current practice. Of patients with pernicious anemia, 90% are positive for these antibodies. However, antiparietal cell antibodies are also present in patients with thyroid disease and other autoimmune disorders.
    - Anti-IF antibodies (type I and II) are highly specific for pernicious anemia. However, tests for these are rarely ordered to diagnose or treat patients with megaloblastosis.
- **Tests for folate deficiency**
    - Serum folate is the earliest indicator of folate deficiency. Serum samples should be collected prior to therapy or transfusions. If necessary, serum can be frozen until the laboratory can perform the test. Folate levels respond rapidly to changes in dietary folate. A low folate level reflects dietary intake during the previous 2-3 days. Conversely, a single meal with normal folate content can restore serum folate levels to normal.
    - The RBC folate level is usually low in patients with folate deficiency. Folate is incorporated into erythrocytes when they are formed, and folate levels do not fluctuate with changes in diet during the lifespan of the RBC. The RBC folate level may not be low in persons with rapidly developing acute folate deficiency. Another limitation of this test is that RBC folate levels are low in more than 50% of patients with cobalamin deficiency, and this test cannot be used to distinguish between these disorders.

Imaging Studies

- Abdominal x-ray films, upper and lower GI series, and CT scans may be useful for detecting and evaluating blind loop syndromes, strictures, and other gastrointestinal tract abnormalities that may cause a blind loop syndrome.

Other Tests

- Cobalamin deficiency - Detection and evaluation of autoimmune disorders, regional ileitis, fish tapeworm infection, Zollinger-Ellison syndrome, pancreatitis, and myeloproliferative disorders
- Folate deficiency - Detect and evaluate pregnancy, malnutrition, and other complications of sprue, chronic hemolysis, and exfoliative dermatitis
- Tests relevant for the diagnosis and evaluation of inborn errors that cause or are associated with cobalamin or folate deficiency

- Bone marrow aspiration and biopsy results are useful to confirm the diagnosis, to rule out myelodysplasia, and to assess the iron stores. Marrow is cellular with erythroid hyperplasia. Megaloblastic RBC precursors are abundant, and giant metamyelocytes are present. Iron stores may vary from high to low. The bone marrow begins to convert from megaloblastic to normoblastic within 12 hours, and normalization is complete within 2-3 days. Therefore, bone marrow aspiration should be performed as soon as possible and preferably before therapy if the procedure is considered useful for the patient's treatment.

Histologic Findings

Bone marrow is hypercellular. An increase in erythropoietic activity is reflected by a decreased or reversed myeloid-to-erythroid ratio. Erythroid precursors have megaloblastic features in that they are larger than normoblastic cells and they have immature nuclear development. Cytoplasmic maturation is normal, but nuclear remnants, Howell-Jolly bodies, may be present in the cytoplasm. Giant bands (neutrophils) may be present. Megakaryocytes may be large and hyperlobulated. Iron stores vary from being increased before therapy to decreased if iron is consumed during therapy for megaloblastosis. Bone marrow studies should be performed before therapy because therapy may restore normoblastic erythropoiesis rapidly.

CONCLUSION

The essential function of a haematology laboratory is to obtain reliable and reproducible data for health screening and epidemiological studies and to provide clinicians with timely, unambiguous, and meaningful results to assist in diagnosing disease and monitoring its response to treatment (Dacie *et al.*, 2006). These objectives must be emphasised in Nigeria with respect to underdiagnosed diseases such as megaloblastic anaemia to improve health care delivery in our changing society.

RECOMMENDATIONS

In order to tackle the challenges of the laboratory diagnosis of megaloblastic anaemia in Nigeria, the following are suggested:

i. Training and retraining of medical laboratory personnel and consultant haematologists/physicians interpreting haematological results

ii. Incorporating detailed haematological tests in the curriculum of undergraduate students and enforcing compliance

iii. Provision of adequate infrastructure, equipment and reagents in haematology laboratories at all levels of primary health care delivery

iv. Use of standard operating procedures and the enforcement quality assurance and quality control measures

v. Characterization of all types of anaemia before the administration of blood, blood products, drugs or haematinics.

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