# Objectivity and Practice of Peripheral Blood Smear Examination for Childhood Anemia Diagnosis in Benghazi Children Hospital /Libya

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## Abstract

*Introduction:* Anemia is a common disease in children. Peripheral Blood Smear (PBS) require effort and human resource. In this study, we will evaluate objectivity of PBS ordering practice in Benghazi Children Hospital/Libya.

*Method:* A retrospective study of PBS reports of 318 child in 2015 was conducted. Data collected include cause of PBS request, along with other relevant demographic, clinical and laboratory details. Kappa statistic was used for analyzing degree of agreement of results between PBS and Complete Blood Count (CBC) in anemia diagnosis.

*Results:* Our study showed that Hematologists and 88.7% by general pediatricians requested only 6.9% of PBS. Anemia was main cause of request in 36.2%. Only 33.7 % of OPD cases referred with suspect of anemia were revealed to be anemic upon PBS examination and 45.4 % referred with no suspect of anemia were revealed to be anemic upon examination in total study population, but not in Neonate. In 65.1%, there was an agreement between diagnosis of anemia by CBC and PBS. There was accordance of 76.6% between exclusion of anemia by CBC and PBS. Hypochromic microcytic anemia was diagnosed in 93 cases (29.2%), macrocytic anemia was diagnosed in 5%, and 65.7% were not anemic.

*Conclusions:* PBS examination is none objectively ordered and over utilized by Pediatricians in outpatient settings and for routine practice with less yield. Establishing laboratory guidelines for improving utility of PBS, careful and complete justified requests, discussion with laboratory staff, and continuous auditing practice are noticeable needs in the Benghazi Children Hospital.

**Keywords:** PBS, Blood Smear Anemia, Benghazi, CBC, Hematology, Libya, Neonate, Children.

## Abbreviation:
Peripheral Blood Smear: PBS
Complete Blood Count: CBC
Total Study Population: TSP

## Introduction
Anemia is one of the most prevalent hematological disorders among pediatric population worldwide, with highest prevalence is in preschool-age children (47.4%). It is usually considered as a manifestation for a spectrum of heterogeneous disorders and clinical conditions either affecting blood forming units or systemic disorders [1, 2].

Anemia diagnosis is merely a laboratory diagnosis. Diagnostic tools for anemia basically include hemoglobin estimation and Peripheral blood smear (PBS) examination. PBS is considered as an essential diagnostic skill in Hematology/Oncology medical specialties. Hematologic disorders interpreted and evaluated upon PBS findings as well as the clinical picture [3]. The PBS examination can be requested by clinician or by clinical pathologist. It is relatively an expensive investigation and manpower consuming and must be used rationally in anemia, leukocyte, platelets disorders and some medical and infectious conditions [4]. In this study we will evaluate objectivity and practice of PBS ordering in anemia diagnosis.

## Material and Method
A retrospective study from 1/1/2015 to 31/12/2015, conducted in

Benghazi children hospital in Libya. Three hundred and eighteen PBS reports have been reviewed in the archive of the hospital. Data collected include; registration data, demographic details, cause of requesting PBS, suspected diagnosis to be confirmed, blood count, any other accompanying test result report, previous or known diagnosis, details of PBS report. See copy in appendix.

Anemia diagnosis was attributed to hemoglobin below 11 gm/dl for children under 5 years and below 12 gm/dl for older ones. Statistical analysis was performed using SPSS program version 18. Kappa statistic was used for analyzing degree of agreement between PBS and CBC results. Interpretation of Kappa statistic (kappa agreement value) was according to the following: Value < 0: Less than chance agreement, 0.01–0.20: Slight agreement, 0.21–0.40: Fair agreement, 0.41–0.60: Moderate agreement, 0.61–0.80: Substantial agreement, 0.81–0.99: Almost perfect agreement.

## Result

Sample Characters: Eighty nine percent of children were Libyan with predominance of male 59%. Data regarding age were missing in 26.1% of reports. Largest number of age categories was under five; 32.4% while neonates; 4.1% and teenagers; 5.3% categories were the smallest (See Figure-1A, B, C). Most of referrals requests were sent from Benghazi children hospital 99.3 %. Outpatient departments (OPDs) request for PBS represent 62.6%, and 6 % from Intensive Care Unit (ICU), while 31.1 % from wards (See Figure-1D).
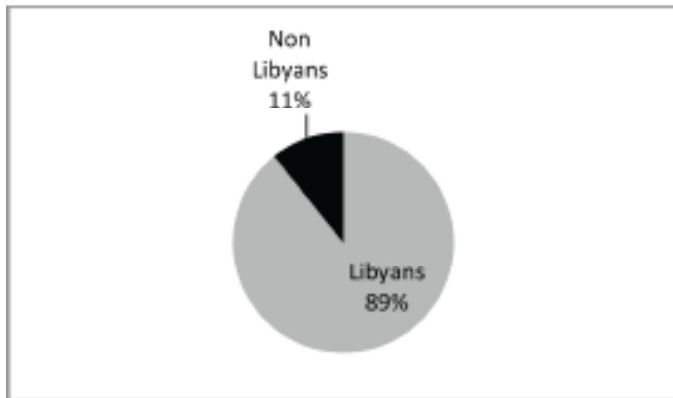


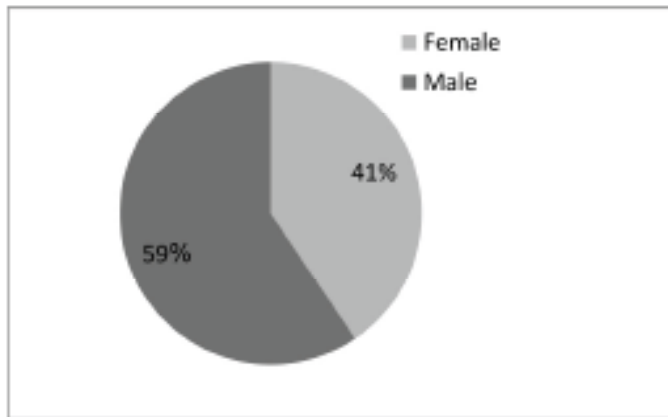**Figure.1A: Nationality of Study Population**
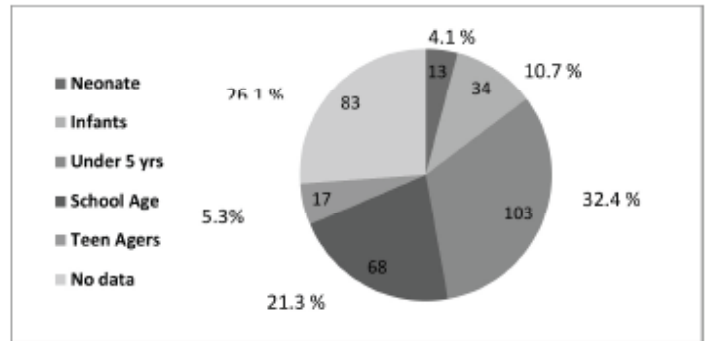


**Figure.1B: Gender of Study Population**



**Figure.1C: Distribution of study population according to age**
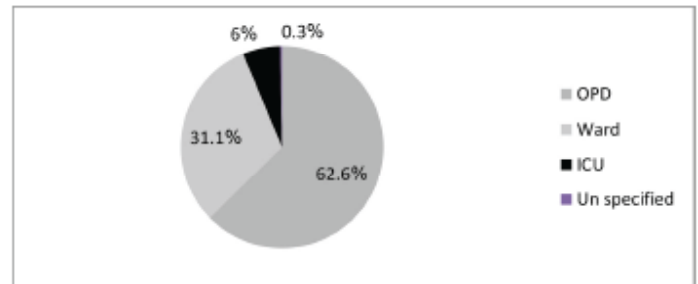


**Figure.1D: Distribution of study population according to setting of referral**

### Refering Doctors and Specification of Anemia and / or Other Underlying Disease in Request:

Most of referring doctors were general pediatricians (88.7%) while hematologists only contributed for (6.9%) of requests (See Table-1A). Anemia was mentioned as the only suspected problem (main cause of the investigation) in 115 cases (36.2%). In 4 cases, anemia was mentioned with another problem. Underlying disease was mentioned only in 165 cases (51.9%). Those included two cases of pancytopenia, two cases of glucose 6-phosphate dehydrogenase enzyme deficiency, one case of iron deficiency anemia, one case of aplastic anemia, one case of sickle cell anemia and one case of Rhesus factor incompatibility. Other underlying diseases included platelet disorders, leukocyte disorders, infections, malignant conditions, polycythemia and miscellaneous medical conditions like brain tumor, cerebral palsy, congenital heart disease, etc (See Table-1B).
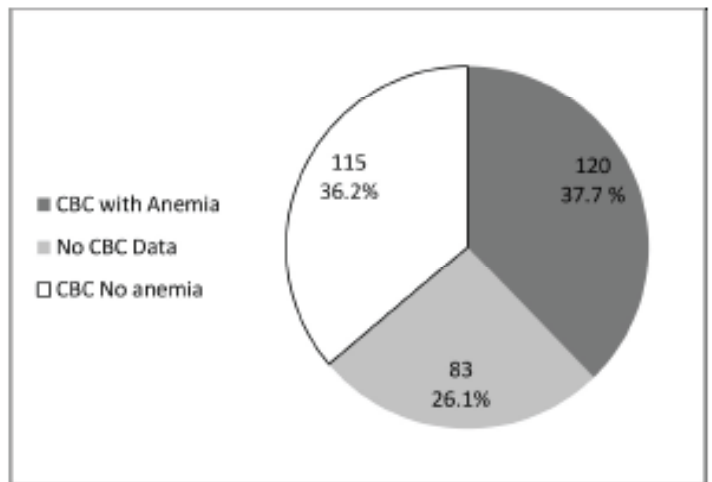
**Table-1A: Distribution of cases according to refering doctors:**

| Referring doctors | Number | Percentage |
|---|---|---|
| Pediatricians | 282 | 88.7% |
| Hematologists | 22 | 6.9% |
| Others | 5 | 1.6% |
| Not Mentioned | 9 | 2.8% |

**Table-1B: Distribution of causes of PBS request according to specification of anemia and/or other underlying complain or**

| Main cause of the PBS request | Number | Percentage |
|---|---|---|
| Anemia as a main cause of request | 115 cases | 36.2% |
| Anemia with other diseases:<br>-Anemia with Leukocytes disorders<br>-Anemia with platelets disorders | 4 cases<br>1 case<br>3 case | 1.2 %<br>0.3%<br>0.9% |
| Cause of request not mentioned | 167 | 52.5 % |
| Other diseases as cause of request:<br>-Platelets problems<br>-Pancytopenia<br>-Leukocytes problems | 32<br>8<br>6<br>18 | 10 %<br>2.5 %<br>1.9%<br>5.7% |

Diagnosis of Anemia by CBC: Mean blood hemoglobin was 10.8 gm/dl (95% CI: 10.6 – 11.1 gm/dl). More than quarter of cases had no CBC result. From total available CBC results (235 cases), 51.06 % (120/235) have anemia, while 48.9% (115/235) have no anemia (See Figure 2A, B). Proportion of diagnosis of anemia by CBC was homogeneous across gender, age and referring doctor categories (P= 0.21, 0.871 and 0.845 respectively). Diagnosis of anemia by CBC was significantly less among OPD cases (30.2%) and routine practice (30.1%) requests respectively, (P<0.001 for both) (See Tables-5).



**Figure-2A: Distribution of total study population (TSP) 318 cases according to avialability of CBC and diagnosis of anemia by CBC.**
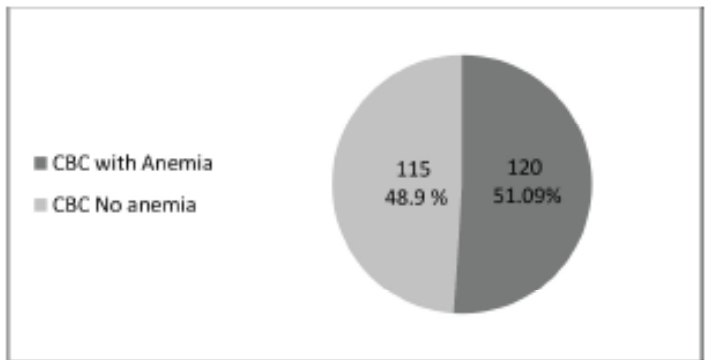


**Figure-2-B: Distribution of anemia cases diagnosed by CBC among cases with available CBC ( 235 cases).**

**Table-2 : Proportion of diagnosis of anemia by CBC from OPD and from routine practice requests.**

| OPD | Anemia by CBC | | Total | Routine | Anemia by CBC | | Total |
|---|---|---|---|---|---|---|---|
| | Yes | No | | | Yes | No | |
| Yes | 60 | 139 | 199 | Yes | 66 | 153 | 219 |
| | 30.2% | 69.8% | 100.0% | | 30.1% | 69.9% | 100.0% |
| No | 60 | 59 | 119 | No | 53 | 44 | 97 |
| | 50.4% | 49.6% | 100.0% | | 54.6% | 45.4% | 100.0% |
| Total | 120 | 198 | 318 | Total | 119 | 197 | 316 |
| | 37.7% | 62.3% | 100.0% | | 37.7% | 62.3% | 100.0% |
| Pearson Chi-Square13.022P<0.001 | | | | Pearson Chi-Square = 17.191, P<0.001 | | | |

Diagnosis of Anemia by Clinical Suspicion and PBS: Clinical suspicion of anemia was significantly less frequent among OPD and routine practice requests. Only one third of OPD cases (33.7 %) referred with suspect of anemia were revealed anemic upon PBS examination. About two third of OPD cases (66.3%) referred with no suspect of anemia were revealed to be anemic upon PBS examination. Routine cases referred with no suspect of anemia (71.7 %) were revealed anemic upon PBS examination. More than quarter of routine cases (28.3%) referred with suspect of anemia were revealed to be anemic upon PBS examination (Table-3).

**Table -3 : Proportion of diagnosis of anemia by clinical suspicion from OPD and routine practice request, role of PBS in childhood anemia:**

| OPD | Anemia is Suspected or Underlying cause | | Total | Routine | Anemia is Suspected or Underlying cause | | Total |
|---|---|---|---|---|---|---|---|
| | Yes | No | | | Yes | No | |
| Yes, as confirmed by PBS | 67 | 132 | 199 | Yes, as confirmed by PBS | 62 | 157 | 219 |
| | 33.7% | 66.3% | 100.0% | | 28.3% | 71.7% | 100.0% |
| No, as reported by PBS | 54 | 65 | 119 | No, as reported by PBS | 59 | 38 | 97 |
| | 45.4% | 54.6% | 100.0% | | 60.8% | 39.2% | 100.0% |
| Total | 121 | 197 | 318 | Total | 121 | 195 | 316 |
| | 38.1% | 61.9% | 100.0% | | 38.3% | 61.7% | 100.0% |
| Pearson Chi-Square = 4.332, P= 0.037. Pearson Chi-Square = 10.354, P= 0.001 | | | | | | | |

Hematological Finding in PBS: Examination of PBS revealed anemia in 129 cases. Hypochromic microcytic anemia in 93 cases, macrocytic anemia was diagnosed in 16 cases, normocytic normochromic anemia in 19 cases and sickle cells seen in 1 case and representing respectively (29.2%), (5%), (6% ) (0.3%) of TSP.

PBS showed target cells in 2 cases (0.6%), heinz bodies in 1 case (0.3%). Lymphoblasts were seen in 6 cases (1.9%) and other lymphocyte abnormalities were seen in 58 cases (18.2%). Monocyte (non-blast abnormalities were seen in 9 cases (2.8%). Eosinophilia was diagnosed in 17 cases (5.3%). Thrombocytopenia was diagnosed in 6 cases (1.9%). In only 89 / 93 cases of microcytic hypochromic anemia, iron study was recommended while in only 4 / 16 of cases with macrocytic anemia, vitamin B12 essay was recommended.

Test Validation of Peripheral Blood Smear: Agreement analysis of PBS and CBC in diagnosis of anemia in the total study population (TPS) is fair to moderate (Table-4). Agreement analysis of PBS and CBC in diagnosis of anemia among each group are seen in (Tables-5).

**Table -4: Agreement of PBS and CBC in diagnosis of Anemia among TPS.**

| Anemia by PBS | Anemia by CBC | | Total |
|---|---|---|---|
| | Yes | No | |
| Yes | 71 | 38 | 109 |
| | 65.1% | 34.9% | 100.0% |
| No | 49 | 160 | 209 |
| | 23.4% | 76.6% | 100.0% |
| Total | 120 | 198 | 318 |
| | 37.7% | 62.3% | 100.0% |

**Table -5: Agreement of PBS and CBC in diagnosis of Anemia among each group:**

| ANEMIA BY PBS | ANEMIA BY CBC | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neonates | | Total | Infants | | Total | Under Five | | Total | School Children | | Total | Teenagers | | Total |
| | Yes | No | | Yes | No | | Yes | No | | Yes | No | | Yes | No | |
| Yes | 3 | 5 | 8 | 12 | 0 | 12 | 32 | 7 | 39 | 17 | 0 | 17 | 7 | 0 | 7 |
| | 37.5% | 62.5% | 100% | 100% | 0.0% | 100% | 821.% | 17.9% | 100.% | 100.% | 0.0% | 100.0% | 100.0% | 0.0% | 100.0% |
| No | 3 | 2 | 5 | 13 | 9 | 22 | 13 | 51 | 64 | 19 | 32 | 51 | 1 | 9 | 10 |
| | 60.0% | 40.0% | 100.0% | 59.1% | 40.9% | 100.0% | 20.3% | 79.7% | 100.0% | 37.3% | 62.7% | 100.% | 10% | 90.% | 100.0% |
| Total | 6 | 7 | 13 | 25 | 9 | 34 | 45 | 58 | 103 | 36 | 32 | 68 | 8 | 9 | 17 |
| | 46.2% | 53.8% | 100.% | 73.5% | 26.5% | 100.% | 43.7% | 56.3% | 100.0% | 52.9% | 74.1% | 100.% | 47.1% | 52.9% | 100% |

Results of Agreement and validity analysis of PBS and CBC in diagnosis of anemia among TSP and different groups according to age categories are seen in (Tables-6). Neonatal group yield poorest test validity indices, (P = 0.429) and teen agers the highest.

**Table-6: Results of PBS and CBC agreement and validity analysis in the study population and according to age and gender categories:**

| Category | Kappa | P(x²) | SEN | SPEC | PV+ | PV- | TE |
|---|---|---|---|---|---|---|---|
| TSP | 0.407† | < 0.001 | 59.2% | 80.8% | 65.1% | 76.6% | 72.6% |
| Neonate | -0.209‡ | 0.429 | 50.0% | 28.6% | 37.5% | 40.0% | 38.5% |
| Infant | 0.328* | 0.01 | 48.0% | 100.0% | 100.0% | 40.9% | 61.8% |
| Under five | 0.599† | <0.001 | 71.1% | 87.9% | 82.1% | 79.7% | 80.6% |
| School age | 0.457† | <0.001 | 47.2% | 100.0% | 100.0% | 62.7% | 72.1% |
| Teenager | 0.881** | <0.001 | 87.5% | 100.0% | 100.0% | 90.0% | 94.1% |
| Male | 0.344* | <0.001 | 56.1% | 78.0% | 57.8% | 76.8% | 70.4% |
| Female | 0.495† | <0.001 | 63.0% | 85.3% | 75.6% | 76.2% | 76.0% |

SEN=sensitivity, SPEC=specificity, PV+=predictive value for positive test, PV-=predictive value for negative test, TE= test efficiency, TSP= Total study population * Fair agreement, † Moderate agreement, ** Substantial agreement ‡Less than chance (poor) agreement.

## Discussion

Some researches indicate that leukocyte related disorders are the most likely cause of PBS order by clinicians, followed by platelets then red blood cells (RBC) abnormalities [5]. However, in this study, our data showed that most of cases are referred for routine workup or for anemia and indicated non-conformity to guidelines and protocols.

The use of PBS examination for review of diagnosis in OPD for instance was found to be needed in only very small percentages if good history data were properly collected [6]. In our study, most of cases were referred from OPD without complete request form or clinical data or history. In this regards, the high request rate of PBS in our study could be attributed to loss of clinical data. They use of PBS as a compensatory tool for deficient data. This practice should be prohibited along with emphasis on importance of patient history and demographic data in our practice.

Most of requests; 62.6% came from outpatient departments (OPDs). Cause of request was just as a part of routine workup in majority of cases; 68.9%. Confirmation of a specific diagnosis requests was aimed in 19.5% of TSP. Most of referring doctors were general pediatricians; 88.7% while hematologists only contributed for 6.9% of requests. This could reflect the professionalism of hematologists.

Diagnosis of anemia by CBC was significantly less among OPD cases and among routine practice requests (P<0.001 for both). Proportion of diagnosis of anemia by PBS was significantly lower among routine practice requests (P <0.001). Those findings support the conclusions of Froom P et al as PBS should be kept in a small percentage of patients as good history and examination should take place [6, 7].

Lack of administrative data was found in 0.3% in place of request (setting), 3.5% in cause of requesting and 2.8% in the specialty of requesting doctor. There was no documented blood group, Rhesus factor and race in any of reviewed report. Data of age were missing in 26.1% of reports. According to Bain BJ 2005, clinical details in requests are of paramount importance in diagnostic utility of PBS and should be provided for laboratory staff [4].

Agreement analysis of PBS and CBC in diagnosis of anemia showed that the agreement in the TPS is fair to moderate, very poor among neonates and highest among teenagers. Neonatal group yield poorest test validity indices and complete homogeneity of cells (P = 0.429). While higher rates of agreements was found with teenage category, with highest agreement and full specificity (100%), positive predictive value (100%), and high-test efficiency (94.1%). This suggest that PBS might be more preferred in certain age groups like teenagers and school age children in diagnosis of anemia. Our result on neonate age group needs to be validated with sufficient number of cases.

Neonatal period and infancy may need targeted investigation rather than overutilization of resources available for PBS. Clinical guidelines and ordering of laboratory tests in algorithmic way could regulate the practice of requesting PBS [4, 5].

PBS examination can be a useful test for investigating and classifying anemia in teenagers and children beyond infancy period. However, it is over utilized in outpatient settings and for routine practice with less yield.

## Conclusion

We should educate the staff, and impose a practical laboratory guideline for anemia diagnosis for non-specialists [8, 9]. Anemia is not a diagnosis in itself, but merely an objective sign of the presence of disease [10]. Evaluation of Anemia requires brief history and physical examination along with laboratory tests [11]. It seems to be much better to keep the use of this test for specialty-based practice, along with strict follow-up of guidelines. Further research and auditing practice of PBS examination is needed in the hospital.

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