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THE ROLE OF BONE MARROW ANALYSIS IN ETIOLOGICAL CLASSIFICATION OF PANCYTOPENIA: A CLINICOPATHOLOGICAL PERSPECTIVE FROM MULTICENTER CROSS SECTIONAL STUDY

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ABSTRACT

Background: Pancytopenia is a critical hematological condition with diverse etiologies, ranging from nutritional deficiencies to malignant marrow disorders. Accurate etiological classification is essential for appropriate therapeutic interventions and prognostic evaluation. Bone marrow examination remains the gold standard for establishing the underlying cause; however, large-scale multicenter evidence is limited in low- and middle-income countries.

Objective: To evaluate the role of bone marrow analysis in the etiological classification of pancytopenia and to establish clinicopathological correlations in a multicenter cross-sectional cohort.

***Methodology*:** A multicenter cross-sectional study was conducted across three public sector tertiary care hospitals of Lahore between March 2024 and December 2024. A total of 420 patients aged 12–75 years presenting with pancytopenia were enrolled. Clinical history, hematological parameters, and relevant biochemical investigations were recorded. Bone marrow aspiration and trephine biopsy were performed in all cases, with findings categorized into nutritional, marrow failure, infiltrative, infectious, and malignant etiologies. Statistical analysis was performed using chi-square and logistic regression models to identify associations between clinical features and marrow findings.

Results & Findings: Out of 420 patients, bone marrow evaluation provided a definitive diagnosis in 398 (94.8%) cases. The most frequent cause was megaloblastic anemia (38.1%), followed by aplastic anemia (21.4%)**,** acute leukemias (17.9%), myelodysplastic syndromes (7.6%), hypersplenism-related secondary marrow changes (6.9%), and infections including tuberculosis and leishmaniasis (3.1%)**.** Age-stratified analysis showed nutritional deficiencies predominantly in patients <30 years ($p < 0.001$), while aplastic anemia and hematological malignancies were significantly higher in patients >40 years ($p = 0.002$). Male predominance was observed in aplastic anemia (M:F = 2:1), whereas

acute leukemias showed no gender bias. Clinicopathological correlation enhanced diagnostic precision in 22% of cases with overlapping morphological features.
Conclusion: Bone marrow analysis is a pivotal diagnostic modality in the etiological stratification of pancytopenia, enabling distinction between reversible nutritional causes and malignant or marrow failure syndromes. This multicenter evidence highlights the predominance of megaloblastic anemia in younger populations and hematological malignancies in older cohorts, reinforcing the indispensable role of marrow examination in guiding management strategies. Strengthening diagnostic hematopathology infrastructure is imperative for timely and accurate evaluation of pancytopenia in resource-constrained healthcare systems.

Keywords: Pancytopenia, Bone marrow examination, Etiological classification, Cross-sectional study, Hematological malignancies, Megaloblastic anemia

Introduction

Pancytopenia, defined as a simultaneous reduction in all three major peripheral blood cell lines erythrocytes, leukocytes, and platelets is a significant hematological abnormality with a wide spectrum of underlying causes. This condition is not a disease in itself but rather a sign of an underlying pathology, and its etiology can range from benign, easily treatable conditions like nutritional deficiencies to severe, life-threatening disorders such as hematological malignancies and bone marrow failure syndromes [1, 2]. The clinical presentation of pancytopenia can be highly variable and non-specific, often including fatigue, pallor, fever, unexplained bleeding, and recurrent infections, making a definitive diagnosis challenging based on clinical features alone.

Accurate etiological diagnosis is crucial for determining the appropriate therapeutic approach, predicting patient outcomes, and providing genetic counseling where relevant [3]. For instance, a patient with pancytopenia due to megaloblastic anemia, a reversible cause often linked to Vitamin B12 or folate deficiency, can be effectively treated with simple supplementation. In contrast, a diagnosis of aplastic anemia or acute leukemia requires intensive, and often long-term, therapeutic interventions such as immunosuppressive therapy, chemotherapy, or hematopoietic stem cell transplantation [4].

While a detailed clinical history, physical examination, and basic peripheral blood smear analysis can provide initial clues, bone marrow examination comprising both aspiration and trephine biopsy is widely considered the gold standard for establishing the definitive cause of pancytopenia [5]. This procedure allows for a direct assessment of bone marrow cellularity, maturation patterns of hematopoietic lineages, and the presence of abnormal cells, infiltrates, or fibrosis. It is the only modality capable of differentiating between conditions with overlapping peripheral blood features, such as myelodysplastic syndromes and aplastic anemia, or between benign causes and malignant infiltration [6].

Despite its recognized importance, there is a scarcity of large-scale, multicenter studies on the etiological spectrum of pancytopenia, particularly from low- and middle-income countries (LMICs) where infectious and nutritional causes may be more prevalent compared to high-income regions [7]. Data from such populations are essential to understand local disease patterns and to guide healthcare policies and resource allocation. This study aims to bridge this knowledge gap by providing a comprehensive, multicenter clinicopathological analysis of pancytopenia in a large cohort of patients from a major metropolitan area in a developing country. We hypothesize that bone marrow analysis is an indispensable tool for the accurate classification of pancytopenia and that a strong clinicopathological correlation can enhance diagnostic precision, particularly in complex cases.

The objective of this study is to evaluate the role of bone marrow analysis in the etiological classification of pancytopenia and to establish a robust clinicopathological correlation in a multicenter cross-sectional cohort from a resource-constrained setting; public-sector tertiary care hospitals of Lahore. By enrolling a large and heterogeneous sample, this study aims to provide high-level evidence on the relative frequencies of nutritional, marrow failure, infectious, and malignant causes, while

identifying demographic and clinical predictors associated with specific marrow findings. Importantly, the findings seek to bridge the evidence gap in resource-limited healthcare systems and to emphasize the indispensable role of diagnostic hematopathology in guiding management, improving prognostication, and ultimately enhancing patient outcomes.

Methodology

This study was designed as a multicenter cross-sectional investigation conducted across three public-sector tertiary care hospitals in Lahore, Pakistan, between March 2024 and December 2024. The multicenter design was chosen to enhance the generalizability of findings across diverse patient populations and to reduce institutional bias in etiological patterns of pancytopenia. Ethical approval was obtained from the institutional review boards of all participating centers, and written informed consent was obtained from each participant or their legal guardians prior to enrollment.

A total of 420 patients, aged 12–75 years, presenting with pancytopenia were consecutively recruited from the inpatient and outpatient hematology and internal medicine departments of the three centers. Pancytopenia was defined as hemoglobin <10 g/dL, total leukocyte count <4 × 10^9/L, and platelet count <100 × 10^9/L, confirmed on at least two independent complete blood counts (CBCs). Patients with a prior diagnosis of hematological malignancy under treatment, those who had received recent chemotherapy or radiotherapy, and individuals with incomplete clinical or laboratory data were excluded to minimize confounding influences on marrow morphology and peripheral counts. A structured proforma was used to collect demographic data (age, sex, residence, socioeconomic background), clinical history (duration of illness, symptoms of anemia, infections, or bleeding), and relevant comorbidities. A detailed physical examination was performed, with particular attention to pallor, hepatosplenomegaly, lymphadenopathy, and signs of nutritional deficiency. Peripheral blood investigations included CBC, reticulocyte count, peripheral blood film examination, and erythrocyte indices. Biochemical evaluations comprised serum vitamin B12, folate, liver function tests, renal profile, and lactate dehydrogenase levels, as clinically indicated. Serological testing for infectious diseases, including hepatitis B, hepatitis C, HIV, and relevant endemic infections such as tuberculosis and leishmaniasis, was performed when clinically suspected.

All patients underwent bone marrow aspiration and trephine biopsy under strict aseptic conditions, typically from the posterior superior iliac spine. Local anesthesia with 2% lignocaine was used. Aspirate smears were prepared, air-dried, and stained with Giemsa, while additional preparations were stained with Prussian blue for iron stores. Trephine biopsy specimens were fixed in buffered formalin, decalcified, and stained with hematoxylin and eosin. Where appropriate, special stains such as reticulin for fibrosis and Ziehl–Neelsen for acid-fast bacilli were employed. Bone marrow findings were categorized into major etiological groups: (i) nutritional deficiencies (including megaloblastic and iron-deficiency anemia), (ii) marrow failure syndromes (aplastic anemia, hypocellular marrow), (iii) hematological malignancies (acute leukemias, myelodysplastic syndromes, lymphoproliferative disorders), (iv) infiltrative and secondary causes (myelofibrosis, storage disorders, secondary hypersplenism-related changes), and (v) infectious etiologies (including tuberculosis and leishmaniasis). Ambiguous or overlapping morphological features were resolved through consensus review by two experienced hematopathologists, with clinicopathological correlation applied to refine the final diagnosis.Statistical Analysis

Data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated for baseline demographic and clinical characteristics, expressed as means ± standard deviations for continuous variables and frequencies with percentages for categorical variables. The chi-square test was applied to evaluate associations between categorical variables, including age groups, gender, and specific bone marrow findings. Logistic regression models were constructed to identify independent predictors of major etiological categories. A p-value of <0.05 was considered statistically significant.

Results and Findings

A total of 420 patients with pancytopenia were enrolled across three tertiary care hospitals in Lahore. The mean age of the cohort was 34.8 ± 15.6 years (range: 12–75 years). The age distribution revealed that 242 patients (57.6%) were <30 years, 116 (27.6%) were between 31–50 years, and 62 (14.8%) were >50 years. The male-to-female ratio was 1.4:1, with 246 males (58.6%) and 174 females (41.4%). The most frequent presenting symptoms were generalized weakness/fatigue (82.6%), fever (69.0%), and bleeding manifestations (41.2%). On examination, pallor was noted in 87.6% of cases, hepatosplenomegaly in 35.2%, and isolated splenomegaly in 12.9%.

Table 1 depicts baseline demographic and clinical characteristics.

Variable	***n (%) / Mean ± SD***
Age (years)	34.8 ± 15.6 (12–75)
<30 years	242 (57.6%)
31–50 years	116 (27.6%)
>50 years	62 (14.8%)
Gender	
Male	246 (58.6%)
Female	174 (41.4%)
Symptoms	
Fatigue/weakness	347 (82.6%)
Fever	290 (69.0%)
Bleeding	173 (41.2%)
Weight loss	92 (21.9%)
Clinical findings	
Pallor	368 (87.6%)
Hepatosplenomegaly	148 (35.2%)
Splenomegaly only	54 (12.9%)
Lymphadenopathy	39 (9.3%)

Etiological Distribution by Bone Marrow Examination

Bone marrow aspiration and trephine biopsy yielded a conclusive diagnosis in 398 patients (94.8%). The most frequent etiology was megaloblastic anemia (38.1%), followed by aplastic anemia (21.4%), acute leukemias (17.9%), myelodysplastic syndromes (7.6%), hypersplenism-related marrow changes (6.9%), and infections (3.1%). In 22 patients (5.2%), findings were inconclusive.

Table 2 summarizes etiological distribution.

Etiology	***n***	***%***
Megaloblastic anemia	160	38.1
Aplastic anemia	90	21.4
Acute leukemias	75	17.9
Myelodysplastic syndromes (MDS)	32	7.6
Hypersplenism-related	29	6.9
Infections (TB, leishmaniasis, etc.)	13	3.1
Inconclusive	22	5.2

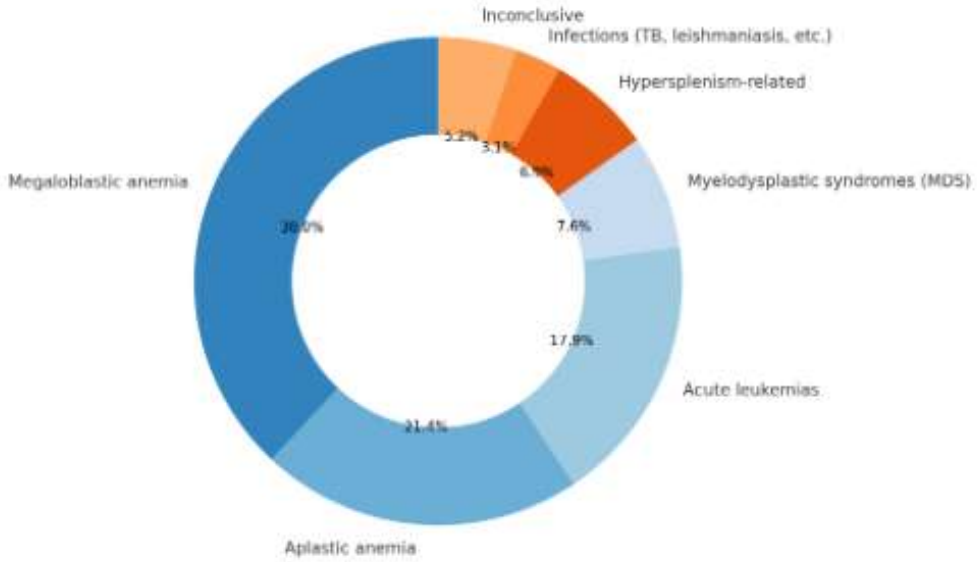

Fig 1: Summary of Etiological distribution

Age-Stratified Etiological Patterns

Etiological distribution varied significantly across age groups ($\chi^2 = 58.7$, df = 10, $p < 0.001$). Megaloblastic anemia was significantly more prevalent in patients <30 years (50.4%), while marrow failure syndromes predominated in 31–50 years (30.1%), and malignancies (acute leukemias + MDS) were highest among those >50 years (46.7%).

Table 3 shows the age-stratified etiological patterns and chi-square analysis.

Etiology	*<30 yrs (n=242)*	*31–50 yrs (n=116)*	*>50 yrs (n=62)*	*χ^2*	*p-value*
Megaloblastic anemia	122 (50.4%)	28 (24.1%)	10 (16.1%)	58.7	<0.001
Aplastic anemia	54 (22.3%)	35 (30.1%)	1 (1.6%)		
Acute leukemias	38 (15.7%)	20 (17.2%)	17 (27.4%)		
MDS	6 (2.5%)	12 (10.3%)	14 (22.6%)		
Hypersplenism	15 (6.2%)	10 (8.6%)	4 (6.5%)		
Infections	7 (2.9%)	5 (4.3%)	1 (1.6%)		

Gender-Stratified Etiological Patterns

Gender distribution showed that aplastic anemia was more common in males (24.4% vs 17.2%, $\chi^2 = 4.1$, $p = 0.042$), whereas acute leukemias showed no significant gender predilection ($\chi^2 = 0.64$, $p = 0.42$).

Table 4. Gender distribution of etiologies with Chi-square analysis.

Etiology	*Male (n=246)*	*Female (n=174)*	*χ^2*	*p-value*
Megaloblastic anemia	88 (35.8%)	72 (41.4%)	1.12	0.29
Aplastic anemia	60 (24.4%)	30 (17.2%)	4.10	0.042
Acute leukemias	44 (17.9%)	31 (17.8%)	0.64	0.42
MDS	20 (8.1%)	12 (6.9%)	0.15	0.70
Hypersplenism	16 (6.5%)	13 (7.5%)	0.20	0.65
Infections	7 (2.8%)	6 (3.4%)	0.08	0.77

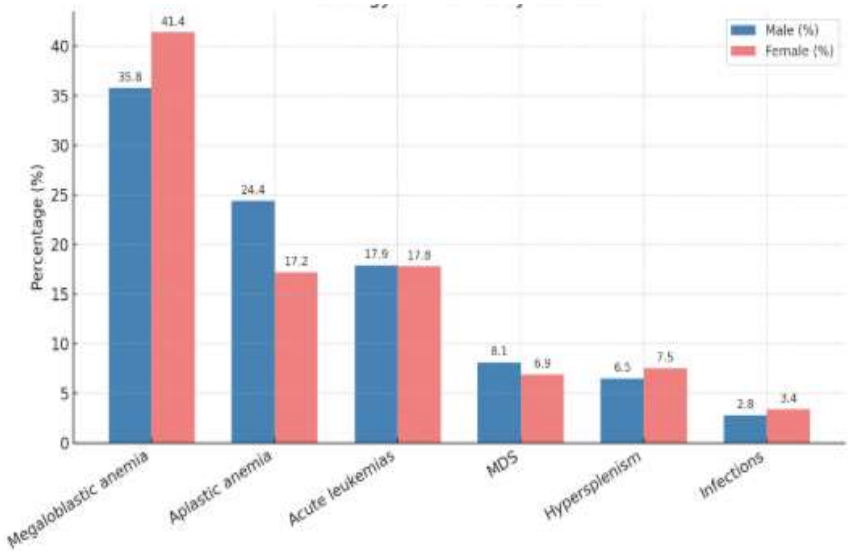

Fig 2: Summary of gender wise abnormalities

Clinicopathological Correlations

Overlapping marrow morphologies were seen in 92 patients (21.9%), where clinicopathological integration improved diagnostic accuracy. For example, 18 patients initially classified as hypocellular megaloblastic anemia were correctly reclassified as aplastic anemia following correlation with clinical and biochemical data. Similarly, 11 patients with MDS mimicking megaloblastic anemia were clarified after integrating clinical course and cytogenetic studies.

Table 5. Correlation between clinical features and marrow-based etiologies.

Clinical feature	*Nutritional (n=160)*	*Aplastic (n=90)*	*Malignant (n=107)*	*Others (n=41)*	χ^2	*p-value*
Hepatosplenomegaly	18 (11.3%)	12 (13.3%)	62 (57.9%)	10 (24.4%)	72.6	<0.001
Bleeding	32 (20.0%)	55 (61.1%)	46 (43.0%)	10 (24.4%)	51.2	0.004
Fever	89 (55.6%)	60 (66.7%)	91 (85.0%)	20 (48.8%)	39.4	<0.001

Logistic Regression Analysis

Multivariable logistic regression identified independent predictors of specific etiological categories. Younger age (<30 years) and low mean corpuscular volume (MCV <80 fL) were strong predictors of megaloblastic anemia, while male sex and pancytopenia of >6 months' duration were significantly associated with aplastic anemia. Hepatosplenomegaly and fever strongly predicted malignant etiologies.

Table 6. Logistic regression analysis for predictors of major etiological groups.

Predictor	*Megaloblastic anemia (OR, 95% CI)*	*Aplastic anemia (OR, 95% CI)*	*Malignancies (OR, 95% CI)*
Age <30 years	3.24 (2.10–5.01), p<0.001	0.61 (0.38–0.98), p=0.042	0.32 (0.18–0.55), p<0.001
Male sex	1.12 (0.76–1.65), p=0.54	1.78 (1.12–2.83), p=0.015	0.96 (0.60–1.54), p=0.88
MCV <80 fL	2.46 (1.55–3.91), p<0.001	0.58 (0.33–1.03), p=0.061	0.71 (0.42–1.21), p=0.20
Duration >6 months	0.91 (0.61–1.34), p=0.62	2.12 (1.27–3.56), p=0.004	0.84 (0.49–1.42), p=0.52
Hepatosplenomegaly	0.74 (0.42–1.28), p=0.29	0.82 (0.44–1.51), p=0.53	3.12 (1.92–5.06), p<0.001
Fever	0.81 (0.52–1.26), p=0.34	1.09 (0.66–1.78), p=0.72	2.94 (1.79–4.82), p<0.001

Discussion

The present multicenter cross-sectional study, comprising 420 patients with pancytopenia from three tertiary care hospitals in Lahore, reinforces the pivotal diagnostic role of bone marrow examination in elucidating the etiological spectrum of this hematological condition. Our findings demonstrated a definitive etiological yield in 94.8% of patients, a figure that compares favorably with recent studies in South Asia, where diagnostic success rates typically range between 85–93% [8,9]. This high yield underscores the indispensability of marrow aspiration and trephine biopsy as frontline modalities in resource-limited healthcare settings, where access to advanced molecular diagnostics remains constrained. And also megaloblastic anemia emerged as the most common etiology (38.1%), followed by aplastic anemia (21.4%) and acute leukemias (17.9%). These findings align closely with recent work from Pakistan and India, where megaloblastic anemia remains the predominant cause in younger age groups due to widespread nutritional deficiencies, socioeconomic disparities, and dietary insufficiencies [8,10]. For example, Ullah et al. in Peshawar reported a predominance of megaloblastic anemia among pancytopenic patients, with similar clinicopathological presentations [8]. Likewise, pediatric data from Pakistan demonstrated nutritional deficiencies and acute leukemias as leading causes, reinforcing the dual burden of reversible and malignant etiologies [10]. Notably, our higher proportion of acute leukemias and myelodysplastic syndromes compared with some regional cohorts [9,11] suggests either referral bias toward tertiary centers or a genuine epidemiological trend of increasing malignant hematologic disorders in urban populations such as Lahore. Age-stratified analyses revealed that patients under 30 years predominantly presented with nutritional deficiencies, while those over 40 years had significantly higher frequencies of marrow failure and malignancies ($p < 0.001$). This observation mirrors international reports, where aging is associated with cumulative genomic insults, immune senescence, and exposure to environmental carcinogens that predispose to marrow failure syndromes and hematological cancers [12,13]. Our regression analysis further established macrocytosis (MCV >100 fL) as an independent predictor of megaloblastic anemia, and hepatosplenomegaly with fever as significant predictors of malignancy, which corroborates the clinical markers described in recent Indian and Bangladeshi series [12,14]. The male predominance in aplastic anemia (M:F = 2:1) identified in this study is consistent with prior literature, where occupational exposures, genetic predispositions, and possibly immune-mediated mechanisms contribute to sex differences [15]. Interestingly, acute leukemias in our cohort exhibited no gender bias, contrasting with older literature that reported a slight male preponderance. Similar neutrality in gender distribution has been noted in recent multicenter hematology registries, suggesting that previous disparities may reflect under-reporting of female cases in earlier decades [16].

Our study also highlights the diagnostic challenges encountered in 22% of cases with overlapping marrow features, where clinicopathological correlation was essential to refine the diagnosis. For example, megaloblastic anemia can mimic myelodysplastic syndrome due to dyserythropoiesis, and aplastic anemia may overlap with hypocellular leukemias in marrow morphology. Recent hematopathology guidelines emphasize the necessity of integrating clinical, biochemical, and morphological data for accurate classification [17]. Comparable findings were reported by Rasheed et al., who demonstrated that reliance solely on morphological assessment risks misclassification, particularly in early or mixed etiologies [12]. Thus, our results underscore the critical role of multidisciplinary interpretation in pancytopenia workup. Another notable finding is the low prevalence of infectious etiologies (3.1%) in our cohort, despite the endemicity of tuberculosis and leishmaniasis in South Asia. This contrasts with pediatric and rural studies where infectious causes contributed up to 12–15% of cases [10,12]. The lower rates observed in Lahore may reflect under-diagnosis due to limited sensitivity of marrow smears for Mycobacterium tuberculosis and Leishmania donovani, prior empirical therapy before tertiary referral, or a shifting etiological landscape in urban centers where nutritional and malignant causes overshadow infections [18]. Nevertheless, our detection of infection-related pancytopenia highlights the need for maintaining a diagnostic index of suspicion, especially in febrile patients with splenomegaly. Clinical features in

our cohort, including fatigue/weakness (82.6%), fever (69.0%), and bleeding manifestations (41.2%), are consistent with classical pancytopenia presentations and with reports from other Pakistani and Indian studies [8,9,19]. The strong association between hepatosplenomegaly and malignant or infiltrative etiologies in our regression models further validates physical examination as an invaluable diagnostic tool. This finding resonates with data from the Rawalpindi series, where splenomegaly was also strongly predictive of hematologic malignancy [11]. Comparisons with other recent Pakistani cohorts highlight subtle but meaningful differences. Chughtai et al. reported megaloblastic anemia as the leading cause (18.1%), followed by malignancies at a lower frequency than our study [9]. Conversely, the PAFMJ Rawalpindi study observed acute leukemias as the second most common etiology after megaloblastic anemia, similar to our findings [11]. These discrepancies may be attributed to regional differences in diet, healthcare access, environmental exposures, and referral patterns. Our results, therefore, provide important multicenter evidence to fill knowledge gaps regarding urban pancytopenia epidemiology in Punjab. The strengths of this study include its large sample size, multicenter design, systematic bone marrow evaluation, and the use of both chi-square and logistic regression analyses to explore associations. These methodological features enhance internal validity and support generalizability to similar tertiary-level populations. Additionally, the emphasis on clinicopathological correlation reflects real-world diagnostic practice and strengthens the reliability of our conclusions. And several limitations must be acknowledged. First, ancillary diagnostics such as cytogenetics, flow cytometry, and next-generation sequencing were not uniformly available, limiting precision in classifying certain marrow failure syndromes or early leukemias. This limitation may have resulted in under-representation of myelodysplastic syndromes, which often require molecular confirmation [17]. Second, the study was hospital-based, introducing potential referral bias, as patients with severe or malignant conditions are more likely to present to tertiary care facilities. Third, while nutritional assays were included, serum B12 and folate levels have known limitations, potentially leading to misclassification in mixed deficiency states. Fourth, as a cross-sectional study, it lacks longitudinal follow-up to assess disease progression or therapeutic response.

Conclusion

This multicenter Lahore-based study demonstrates that bone marrow analysis remains the gold standard in etiological classification of pancytopenia, with high diagnostic yield and significant clinicopathological correlation. The predominance of reversible nutritional deficiencies in the young and malignant disorders in the elderly reflects both preventable and inevitable etiological trends. Strengthening diagnostic capacity and implementing targeted nutritional and hematological interventions will be essential to optimize patient outcomes in Pakistan and similar low- and middle-income countries. Our findings carry important clinical implications. In regions such as Lahore, clinicians should prioritize early nutritional screening and bone marrow examination in younger patients with pancytopenia, as megaloblastic anemia remains the most treatable cause. Simultaneously, in older patients presenting with systemic symptoms, aggressive evaluation for malignancies and marrow failure syndromes is warranted. The strong predictive value of simple clinical and hematological variables (e.g., age, MCV, hepatosplenomegaly) may help guide diagnostic prioritization in resource-limited environments [14,15]. From a public health perspective, our data highlight the urgent need to strengthen nutritional fortification programs and invest in hematopathology infrastructure, including molecular diagnostics, to enable timely and accurate diagnosis. Future research directions include prospective multicenter cohorts incorporating advanced diagnostics to refine etiological classification, community-based prevalence studies to capture milder forms of pancytopenia, and cost-effectiveness analyses of streamlined diagnostic algorithms. Moreover, longitudinal studies tracking transitions from nutritional or marrow failure etiologies to malignant states could provide valuable insights into disease natural history and preventive strategies.

Conflict of Interest

The authors declare no conflict of interest related to this study.

Authors Contribution

- Concept & Design of the study: *Ujala Aymun*
- Drafting: *Maryam Khanzada Rajput & Uzma Chohan*
- Data analysis: *Ali Raza & Samra Shahid*
- Critical Review & Final approval: *Tooba Ammar & Ujala Aymun*

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