# Comparison of different criteria for the diagnosis of primary myelofibrosis reveals limited clinical utility for measurement of serum lactate dehydrogenase

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## ABSTRACT

Primary myelofibrosis shows histological and pathogenetic overlap with essential thrombocythemia and polycythemia vera. Several diagnostic classifications have been proposed for primary myelofibrosis, although little is known about their clinical utility. In a comparison of three recent classifications, overall concordance was 79%. Inclusion of raised serum lactate dehydrogenase categorized 9% of patients as primary myelofibrosis when other criteria were not met. Although mean serum lactate dehydrogenase levels were higher in patients with primary myelofibrosis, levels were also increased in the majority of patients with essential thrombocythemia or polycythemia vera, and significant overlap was observed. A positive correlation with higher leukocyte and platelet count, and disease duration in primary myelofibrosis, suggests that serum lactate dehydrogenase is a biomarker for disease bulk and/or cellular proliferation. In conclusion, raised lactate dehydrogenase lacks specificity for primary myelofibrosis, consistent with the concept of a phenotypic continuum between essential thrombocythemia, polycythemia vera and primary myelofibrosis.

Key words: myeloproliferative neoplasm, lactate dehydrogenase, primary myelofibrosis, essential thrombocythemia, polycythemia vera.

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## Introduction

The classical myeloproliferative neoplasms (MPN), comprising essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF), have historically been considered as distinct clinical entities. The discovery of an identical *JAK2* V617F mutation in all three disorders has challenged this dogma, and precipitated a re-evaluation of how these conditions are classified.[1,2] *JAK2* V617F-positive ET and PV share many clinical and laboratory features, and may be considered part of a phenotypic continuum.[3] PMF is a heterogeneous disorder with a worse overall survival than ET or PV.[4] Several different classification systems have been proposed for the diagnosis of PMF, based on bone marrow histology and additional clinical and/or laboratory features. However these classifications use different sets of criteria to reach a diagnosis of PMF, and little is known about inter-classification concordance. We have, therefore, used a cohort of well-characterized patients to undertake a comparison of three sets of criteria widely used in the diagnosis of PMF and to investigate the utility of individual criteria in distinguishing PMF from ET and PV.

## Design and Methods

Local Research Ethics Committee approval was obtained and the study was carried out in accordance with the principles of the Declaration of Helsinki. ET and PV were diagnosed according to BCSH criteria.[5,6] The t-test was used for pairwise univariate analysis of continuous variables and linear regression was used to test for correlation between two continuous variables. Statistical and Receiver Operating Characteristic (ROC) analyses were performed using Prism version 5.01.

## Results and Discussion

A comparison was made of three classification systems widely used in the diagnosis of PMF (Table 1). We used our institutional database, which includes comprehensive clinical and laboratory data from patients diagnosed with ET, PV or PMF, to identify 58 patients who fulfilled criteria for PMF according to one or more of the following classifications: the Italian Cooperative Study Group (ICSG),[7] Campbell and Green (C&G)[1] and the World Health Organization 2008 (WHO).[8] Of these 58 patients, 46 (79%) were diagnosed with PMF according to all three classification systems. Concordance was highest between ICSG and C&G (90%), followed by C&G and WHO (86%), then ICSG and WHO (81%) (Figure 1A).

Four patients (7%) met PMF criteria according to C&G and WHO, but not ICSG; all 4 patients lacked a leukoerythroblastic blood film but had bone marrow fibrosis and other features to support a diagnosis of PMF including tear-drop red

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**Table 1.** Classification systems used in the diagnosis of primary myelofibrosis.

| WHO 2008[8] | | Campbell & Green 2006[1] | | Italian Cooperative Study Group 1999[7] | |
|---|---|---|---|---|---|
| *Requires A1-A3 and any two B criteria* | | *A1 + A2 and any two B criteria* | | *A1-A3 and any two B criteria or A1-A2 and any four B criteria* | |
| A1 | Megakaryocyte atypia and fibrosis or megakaryocyte atypia, increased granulcytic and decreased erythroid cellularity without fibrosis | A1 | Reticulin ≥3 (on a 0-4 scale) | A1 | Diffuse bone marrow fibrosis |
| A2 | Not meeting WHO criteria for PV, CML, MDS or other myeloid neoplasm | A2 | Pathogenetic mutation (e.g. in *JAK2* or *MPL*) or absence of *BCR-ABL1* | A2 | Absence of *BCR-ABL1* |
| A3 | Acquired mutation or clonal marker or no reactive cause for fibrosis | | | A3 | Palpable splenomeglay |
| B1 | Leukoerythroblastic blood film | B1 | Palpable splenomegaly | B1 | Tear-drop red cells |
| B2 | Increased lactate dehydrogenase[¶] | B2 | Unexplained anemia[‡] | B2 | Circulating immature myeloid cells |
| B3 | Anemia[¶] | B3 | Tear-drop red cells | B3 | Circulating erythroblasts |
| B4 | Palpable splenomeglay[¶] | B4 | Leukoerythroblastic blood film | B4 | Megakaryocyte atypia |
| | | B5 | Constitutional symptoms[†] | B5 | Extramedullary hematopoiesis |
| | | B6 | Extramedullary hematopoiesis | | |

*[¶]Degree of abnormality may be mild or marked; [‡]hemoglobin <11.5 g/dL for men; <10 g/dL for women; [†]weight loss >10% over 6 months, drenching sweats or diffuse bone pain.*

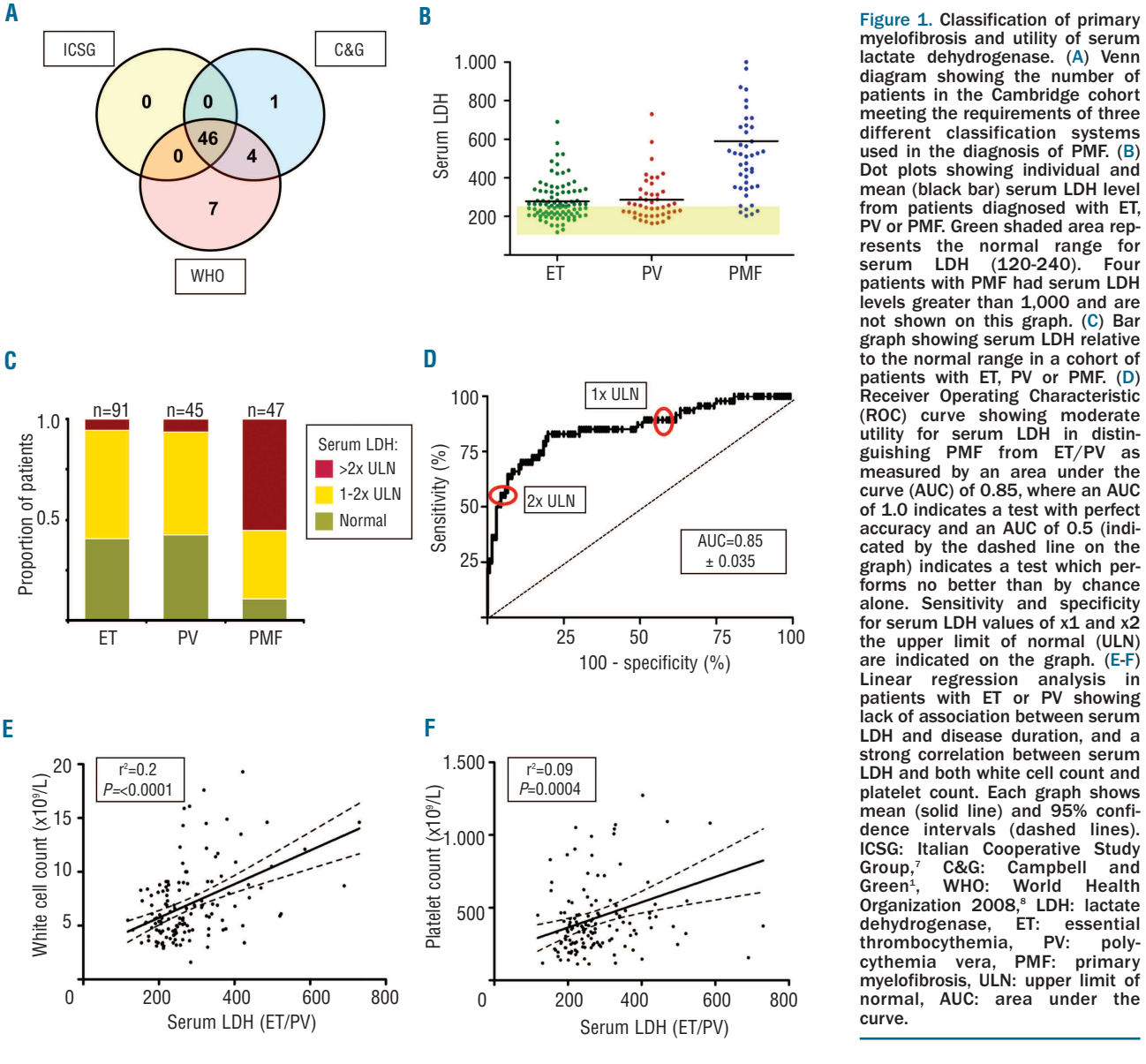


**Figure 1.** Classification of primary myelofibrosis and utility of serum lactate dehydrogenase. (A) Venn diagram showing the number of patients in the Cambridge cohort meeting the requirements of three different classification systems used in the diagnosis of PMF. (B) Dot plots showing individual and mean (black bar) serum LDH level from patients diagnosed with ET, PV or PMF. Green shaded area represents the normal range for serum LDH (120-240). Four patients with PMF had serum LDH levels greater than 1,000 and are not shown on this graph. (C) Bar graph showing serum LDH relative to the normal range in a cohort of patients with ET, PV or PMF. (D) Receiver Operating Characteristic (ROC) curve showing moderate utility for serum LDH in distinguishing PMF from ET/PV as measured by an area under the curve (AUC) of 0.85, where an AUC of 1.0 indicates a test with perfect accuracy and an AUC of 0.5 (indicated by the dashed line on the graph) indicates a test which performs no better than by chance alone. Sensitivity and specificity for serum LDH values of x1 and x2 the upper limit of normal (ULN) are indicated on the graph. (E-F) Linear regression analysis in patients with ET or PV showing lack of association between serum LDH and disease duration, and a strong correlation between serum LDH and both white cell count and platelet count. Each graph shows mean (solid line) and 95% confidence intervals (dashed lines). ICSG: Italian Cooperative Study Group,[7] C&G: Campbell and Green[1], WHO: World Health Organization 2008,[8] LDH: lactate dehydrogenase, ET: essential thrombocythemia, PV: polycythemia vera, PMF: primary myelofibrosis, ULN: upper limit of normal, AUC: area under the curve.

**Table 2.** Sensitivity and specificity of criteria used in the diagnosis of primary myelofibrosis.

| Diagnostic criterion | Classification system | Sensitivity (%) | Specificity *versus* ET and PV |
|---|---|---|---|
| Megakaryocyte atypia | ICSG and WHO | 100 | na |
| Increased bone marrow fibrosis | ICSG and C&G | 97 | 95% |
| Raised serum LDH | WHO | 89 | 40% |
| Tear-drop red cells | ICSG and C&G | 84 | na |
| Anemia (any level) | WHO | 79 | 93% |
| Circulating erythroid progenitors | ICSG | 78 | na |
| Circulating myeloid progenitors | ICSG | 76 | na |
| Leukoerythroblastic blood film | C&G and WHO | 76 | na |
| Palpable splenomegaly | ICSG, C&C and WHO | 63 | 84% |
| Anemia (♂: <11.5g/dL, ♀: <10g/dL) | C&G | 59 | 98% |
| Constitutional symptoms | C&G | 17 | 99% |
| Histological evidence of EMH | C&G and ICSG | 0 | 100% |

*ICSG: Italian Cooperative Study Group,[7] C&G: Campbell and Green,[1] WHO: World Health Organization 2008,[8] LDH: lactate dehydrogenase, EMH: extramedullary hematopoiesis, na: data not available.*

cells and anemia (*Online Supplementary Table S1*). A single patient met only C&G criteria for PMF, on the basis of bone marrow fibrosis, anemia and tear-drop red cells in the absence of splenomegaly or a leukoerythroblastic blood film. Seven patients (12%) met only WHO criteria for PMF. Two of these 7 had sufficient B-criteria to support a diagnosis of PMF according to any of the three classification systems; however, the absence of significant bone marrow fibrosis excluded PMF according to ICSG and C&G criteria. As such, these 2 patients would be classified as 'pre-fibrotic myelofibrosis' according to the WHO system. In the remaining 5 patients (9%), a diagnosis of PMF by WHO criteria was based on bone marrow fibrosis along with mild anemia and raised serum lactate dehydrogenase (LDH). In summary, the overall concordance between the three PMF classification systems was close to 80%. The WHO classification labeled the highest number of patients as having PMF. This was largely due to inclusion of serum LDH in the WHO classification which categorized 5 patients (9%) as having PMF when ICSG or C&G criteria were not met. Despite its inclusion in the WHO criteria, there is surprisingly little published analysis of the utility of serum LDH in the diagnosis of PMF. In view of this, we undertook further investigation of serum LDH in patients with an MPN, and compared its utility to other criteria commonly used in the diagnosis of PMF.

Serum LDH levels were available from 47 patients diagnosed with PMF according to ICSG, C&G and/or WHO criteria, and these were compared to a cohort of patients with ET (n=91) or PV (n=45). Samples were acquired at diagnosis or follow up. Mean serum LDH (normal range 120 - 240) for PMF patients was 591±347, for ET patients 278±100, and for PV patients 286±114. Considerable overlap was observed in LDH levels between patients with ET, PV and PMF (Figure 1B). A serum LDH above the normal range was detected in 89% of patients with PMF, as well as in 60% of ET patients and 58% of those with PV. These data indicate that despite a sensitivity of 89% for the diagnosis of PMF, raised serum LDH performs poorly as a discriminator of PMF from ET or PV, with a specificity of only 40%. By comparison, other criteria performed better at discriminating PMF from ET/PV, with a specificity of 84% for palpable splenomegaly and greater than 90% for constitutional symptoms, anemia or bone marrow fibrosis (Table 2).

Receiver Operating Characteristic (ROC) curve analysis indicated that serum LDH had only moderate utility in the diagnosis of PMF (Figure 1D). In this analysis, a serum LDH of twice the upper limit of normal was identified as a reasonable discriminator of PMF from ET/PV, with a sensitivity of 55% and specificity of 94% (Figure 1C and D). These findings indicate that in contrast to criteria such as palpable splenomegaly, anemia or bone marrow fibrosis, an unspecified increase in serum LDH (as used in the WHO classification) lacks specificity as a diagnostic criterion for PMF, with a raised serum LDH also found in the majority of patients with ET or PV.

Given that serum LDH is raised in the majority of patients with ET, PV or PMF, we performed a further analysis to investigate potential mechanisms underlying this observation. As the mean and range of serum LDH levels were almost identical in ET and PV, these patients were analyzed as a single group. In ET/PV, mean serum LDH level was slightly higher in samples obtained at diagnosis compared to those obtained at follow up (mean LDH level at diagnosis 340±123, mean LDH level at follow up 277±102; $P$=0.08). This difference may well reflect a decrease in serum LDH following treatment with cytoreductive agents, as mean serum LDH level was lower in treated compared to untreated ET/PV patients (mean LDH untreated patients 312 ± 108, mean LDH treated patients 271±102; $P$=0.06). No association was seen between LDH level and *JAK2* V617F mutation status ($P$=0.6). Using linear regression analysis, serum LDH in ET/PV was strongly correlated with both higher white cell count ($P$=<0.0001) and higher platelet count ($P$=0.0004), but showed no association with disease duration ($P$=0.9) (Figure 1E and F). In patients with PMF, serum LDH level did not differ in diagnostic compared to follow-up samples, was not lower in patients receiving cytoreductive therapy, and was not associated with *JAK2* V617F mutation status ($P$>0.5 in all cases). Using linear regression analysis, serum LDH was correlated with both higher white cell count ($P$=0.0001) and longer disease duration ($P$=0.02), but showed no association with platelet count. The strong correlation between serum

LDH and white cell and platelet count in ET/PV and white cell count in PMF suggests that serum LDH serves as a biomarker for disease bulk and/or cellular proliferation in MPN patients.

Although traditionally considered as a distinct clinical entity, it has recently been suggested that PMF frequently represents a pre-existing undiagnosed MPN presenting in accelerated phase.[1] In support of this notion, PMF and myelofibrotic transformation of ET/PV are indistinguishable in phenotype and pattern of cytogenetic and molecular abnormalities.[1, 2, 9-11] Moreover, both are characterized by genomic instability,[11] higher rates of leukemic transformation and shortened overall survival.[12] The increased serum LDH levels in PMF patients may, therefore, reflect increased disease bulk and/or proliferative activity associated with the accumulation of additional genetic lesions during disease acceleration. However, if serum LDH levels were only a marker for disease acceleration then one might expect an association with disease duration in ET and PV; but this was not observed. It, therefore, seems likely that, in the context of an MPN, serum LDH levels are influenced by additional factors such as genetic background. Importantly, our results indicate that an increase in serum LDH level above the normal range is not specific for PMF and should be omitted from future diagnostic classification systems. These findings further emphasize the need for molecular markers to refine disease classification and risk stratification.

## Authorship and Disclosures

*The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.*

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