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# *Primary Myelofibrosis: Serum Lactate Dehydrogenase as a predictor of early Leukemic Progression*

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# *Primary Myelofibrosis: Serum Lactate Dehydrogenase as a predictor of early Leukemic Progression*

ORIGINAL ARTICLE


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**RESUMO**

**Introdução:** A Mielofibrose Primária (MFP) é uma neoplasia mieloproliferativa comumente associada a um prognóstico desfavorável, sendo que a complicação mais alarmante é a sua capacidade de evoluir para Leucemia Mielóide Aguda. A evidência mais recente sugere que níveis séricos aumentados de lactato desidrogenase (LDH), aquando do diagnóstico inicial de MFP, têm uma relação significativa com pior prognóstico e progressão leucémica (PL). No entanto, nunca foi estabelecido um ponto de corte, com significância prognóstica, dos níveis de LDH na MFP.

O nosso estudo visa caracterizar um *cohort* de doentes com MFP, diagnosticados num hospital central de 2010 a 2017, com seguimento até 2022, e investigar uma possível correlação entre níveis aumentados de LDH e PL como evento precoce, *i.e.*, nos primeiros 5 anos após o diagnóstico inicial de MFP. Posteriormente, pretende-se estabelecer o valor de corte com maior significado prognóstico para a prática clínica.

**Materiais e Métodos:** Todos os dados foram recolhidos através de processos clínicos, sendo este estudo retrospetivo, observacional e unicêntrico. A seleção dos participantes, teve por base o seu cumprimento dos critérios de diagnóstico de MFP da Organização Mundial da Saúde, um tempo mínimo de acompanhamento de 5 anos e a disponibilidade das medições de LDH aquando do diagnóstico inicial. Para estudar a relação entre os níveis de LDH ao diagnóstico e PL a 5 anos, foram ainda recolhidas variáveis que atualmente incorporam *scores* de prognóstico. Realizaram-se regressões logísticas, univariável e multivariável, seguidas de uma análise *Receiver Operating Characteristics* (ROC) para avaliar a causalidade entre níveis aumentados de LDH e a probabilidade de PL a 5 anos, estabelecendo posteriormente o ponto de corte mais significante.

**Resultados:** 53 pacientes preencheram os critérios supracitados, com uma idade média de 68 anos, 62,3% do sexo masculino, um nível mediano de LDH de 414 U/L, com PL a 5 anos ocorrendo em 15,1% dos participantes. Em análise univariável, a probabilidade de PL a 5 anos aumentou 0,6% por cada U/L de aumento de LDH [*odds ratio* (OR) 1,006, *p*=0,010]. Em análise multivariável, incluindo parâmetros do *Dynamic Prognostic Scoring System* (DIPSS), o nível de LDH manteve significância (OR 1,007, *p*=0,025). Um valor de corte de 522 U/L foi calculado, com uma sensibilidade e especificidade de 75% e 80%, respetivamente.

**Conclusão:** Demonstrámos uma correlação positiva entre valores aumentados de LDH ao diagnóstico e o desenvolvimento de PL nos primeiros 5 anos, estabelecendo subsequentemente que níveis séricos de LDH ≥522 U/L são preditores independentes de PL a 5 anos.

**PALAVRAS-CHAVE:** Mielofibrose Primária, Lactato Desidrogenase, Fator de Risco, Leucemia Mielóide Aguda

**ABSTRACT**

**Introduction:** Primary Myelofibrosis (PMF) is a myeloproliferative neoplasm commonly associated with an unfavorable prognosis, seeing as the most alarming complication relies on PMF ability to progress to Acute Myeloid Leukemia. The latest evidence suggests that increased levels of serum lactate dehydrogenase (LDH), at the time of initial diagnosis of PMF, have a significant correlation with poor prognosis and leukemic progression (LP). However, no exact cut-off value was ever established for serum LDH usage in PMF prognostication.

Our study aims to characterize a cohort of patients with PMF, diagnosed in a central hospital between 2010 and 2017, with follow-up to 2022, and investigate a possible correlation between increased serum LDH levels and LP as an early event, *i.e.,* within the first 5 years after initial diagnosis of PMF. Subsequently, we intend to establish the optimal cut-off value for clinical practice.

**Materials and Methods:** All data was collected from clinical files, this being a retrospective, observational and single-center study. Patients were selected based on their meeting the World Health Organization criteria for diagnosis of PMF, having a minimum follow-up time of 5 years and the availability of serum LDH level at the time of initial diagnosis. In order to study serum LDH ability to predict LP at 5 years, variables that incorporate prognostic scores were also collected. Univariate and multivariate logistic regression statistics, and a Receiver Operating Characteristics (ROC) analysis curve were performed in order to assess the effect of increased serum LDH on the likelihood of LP in 5 years' time and establish the optimal cut-off point.

**Results:** A total of 53 patients met the above-outlined criteria, having a median age of 68 years, 62.3% males, a median serum LDH level of 414 U/L, with LP within 5 years occurring in 15.1% of patients. In univariate analysis, the likelihood of LP in 5 years' time increased 0.6% by each increasing U/L of serum LDH [odds ratio (OR) 1.006, $p$=0.010]. In multivariate analysis, only including *Dynamic Prognostic Scoring System* (DIPSS) variables, serum LDH retained its significance (OR 1.007, $p$=0.025). A cut-off value for serum LDH was calculated at 522 U/L, with a sensitivity and specificity of 75% and 80%, respectively.

**Conclusion:** We demonstrated a positive correlation between increased serum LDH and LP within the first 5 years after initial diagnosis of PMF, subsequently establishing that serum LDH levels ≥522 U/L independently predict LP in 5 years' time, with the highest achievable sensitivity and specificity.

**Keywords:** Primary Myelofibrosis, Lactate Dehydrogenase, Risk Factor, Acute Myeloid Leukemia

## INTRODUCTION

Primary Myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN), caused by a hematopoietic stem cell defect that is associated with clonal proliferation of megakaryocytes and granulocytes in the bone marrow (BM), culminating in its progressive fibrosis, along with osteosclerosis, ineffective and extramedullary hematopoiesis.[1] PMF has an estimated incidence rate of 0.1-1 cases per 100 000 people per year, occurring more frequently in males than females, with an average age of diagnosis between 69 and 76 years of age.[2]

In the 2016 Myeloid Neoplasm Classification, World Health Organization (WHO) indicates Polycythemia Vera (PV), Essential Thrombocythemia (ET) and PMF as the "*JAK2* MPNs", where PMF is sub-categorized into "prefibrotic/early stage" and "overt fibrotic stage".[3] Recently, the International Consensus Classification (ICC) of myeloid neoplasms and acute leukemias was created, aiming at facilitating diagnosis and prognostication, improving treatment and allowing for innovative clinical trials.[4] Also, in the upcoming 5th edition of the WHO Classification of Hematolymphoid Tumors, minor changes in diagnostic criteria for myeloproliferative neoplasms are expected.[5]

These 3 entities are commonly referred to as the *BCR::ABL1*-negative */Philadelphia*-negative MPNs. PV and ET, in about 15% of cases, may progress to a fibrotic stage over time, achieving clinical and laboratory findings similar to those of PMF, then named secondary myelofibrosis, either post-PV or ET.[3]

Genetic/epigenetic mutations are at the essence of MPN's pathogenesis, and are grouped together under the terms "driver" mutations, involving the *JAK2V617F*, *MPL* and *CALR* genes; and "non-driver" or high molecular risk (HMR) mutations, regarding the *ASXL1, EZH2, SRSF2, IDH1/2, U2AF1* genes*,* amongst many more.[3] In general, 2 in each 3 patients present the *JAK2* gene mutation, but some may not harbor any of the driver mutations, having a "triple-negative" mutational status.[1]

Typical clinical presentation of PMF consists of constitutional symptoms (chronic fatigue, cachexia, night sweats), pruritus, thrombohemorrhagic events, infections, bone pain, hepatosplenomegaly and portal hypertension conducting to variceal bleeding and ascites; and non-hepatosplenic extramedullary hematopoiesis that may originate cord compression and other complications. Laboratory hallmarks of PMF include progressive anemia, leukocytosis or leukopenia, thrombocytosis or thrombocytopenia with circulating megakaryocytes, leukoerythroblastosis [nucleated erythrocytes, teardrop-shaped erythrocytes (dacrocytosis) and immature granulocytes] in the peripheral blood smear and a "dry" tap BM aspirate.[1,3]

Regarding PMF diagnosis, ICC updated diagnostic criteria, similar to those of WHO 2016, are presented in Table 1.[4]

**Table 1.** International Consensus Classification updated diagnostic criteria for PMF

| **Primary myelofibrosis (prefibrotic)**<br><br>(All three major criteria and one minor criterion are needed for diagnosis, confirmed in 2 consecutive determinations) | **Primary myelofibrosis (overt fibrotic)**<br><br>(All three major criteria and one minor criterion are needed for diagnosis, confirmed in 2 consecutive determinations) |
|---|---|
| Major criteria:<br>1. Bone marrow biopsy with megakaryocytic proliferation and atypia, increased age-adjusted bone marrow cellularity, granulocytic proliferation and (often) decreased erythropoiesis, bone marrow fibrosis grade <2<br>2. *JAK2, CALR* or *MPL* mutation, or presence of another clonal marker, or absence of reactive bone marrow reticulin fibrosis<br>3. Not meeting diagnostic criteria for other myeloid neoplasms | Major criteria:<br>1. Bone marrow biopsy with megakaryocytic proliferation and atypia, accompanied by grade ≥2 reticulin/collagen fibrosis<br>2. *JAK2*, *CALR* or *MPL* mutation, or presence of another clonal marker, or absence of reactive bone marrow reticulin fibrosis<br>3. Not meeting diagnostic criteria for other myeloid neoplasms |
| Minor criteria:<br>– Anemia not attributed to other conditions<br>– Leukocytosis ≥11 × $10^9$/L<br>– Palpable splenomegaly<br>– Increased lactate dehydrogenase level | Minor criteria:<br>– Anemia not attributed to other conditions<br>– Leukocytosis ≥11 × $10^9$/L<br>– Palpable splenomegaly<br>– Increased lactate dehydrogenase level<br>– Leukoerythroblastosis |

Table adapted from Arber et al.[4]

Among PMF patients, the most common causes of death are progression into blast phase and acute myeloid leukemia (AML), cardiovascular events and complications of cytopenia, such as infection or bleeding.[3]

Currently, prognostic stratification scores (Table 2)[1] have a major role in the choice of what treatment modality to use, risk-adapted to each patient. Prognostic modelling in myelofibrosis (MF) started in 2009, with the introduction of the International Prognostic Scoring System (IPSS) for PMF, which relies on clinical parameters at diagnosis. Later, dynamic prognostic models (DIPSS, DIPSS-plus) were introduced, which enabled prognostic modelling over the disease's course. Recently, genetic factors were also added to new scoring systems, like the Mutation-enhanced International Prognostic Scoring System for Transplant-age Patients (MIPSS70, MIPSS70-plus, MIPSS70-plus version 2.0) which utilizes karyotype, genetic mutations and clinical data for transplantation-age patients (≤70 years).

One of the latest additions to prognostic scoring in PMF is the Genetically-Inspired Prognostic Scoring System (GIPSS), which is exclusively dependent on karyotype and genetic markers.[1,3,6] All previous models are based in PMF patients, as such, the Myelofibrosis Secondary to PV and ET- Prognostic Model (MYSEC-PM) was developed.[7]

**Table 2.** Current prognostic stratification scores for PMF (IPSS, DIPSS, MIPSS70-plus version 2.0, GIPSS)

| **International Prognostic Scoring System (IPSS)** | **Dynamic International Prognostic Scoring System (DIPSS)** | **Mutation-enhanced International Prognostic Scoring System for Transplant-age Patients (MIPSS70-plus version 2.0)** | **Genetically-Inspired Prognostic Scoring System (GIPSS)** |
|---|---|---|---|
| • Age >65 years (1 point)<br>• Constitutional symptoms (1 point)<br>• Hemoglobin <10g/dL (1 point)<br>• Leukocyte count >25x10$^9$/L (1 point)<br>• Circulating blasts ≥1% (1 point) | • Age >65 years (1 point)<br>• Constitutional symptoms (1 point)<br>• Hemoglobin <10g/dL (2 points)<br>• Leukocyte count >25x10$^9$/L (1 point)<br>• Circulating blasts ≥1% (1 point) | • Severe anemia (2 points)<br>• Moderate anemia (1 point)<br>• Circulating blasts ≥2% (1 point)<br>• Constitutional symptoms (2 points)<br>• Very high risk karyotype (4 points)<br>• Unfavorable karyotype (3 points)<br>• ≥2 HMR mutations (3 points)<br>• One HMR mutation (2 points)<br>• *Type 1/like CALR* absent (2 points) | • Very high risk karyotype (2 points)<br>• Unfavorable karyotype (1 point)<br>• *ASXL1* (1 point)<br>• *SRSF2* (1 point)<br>• *U2AF1Q157* (1 point)<br>• *Type 1/like CALR* absent (1 point) |
| **Low risk:**<br>0 points (135 months)<br>**Intermediate-1 risk:**<br>1 point (95 months)<br>**Intermediate-2 risk**:<br>2 points (48 months)<br>**High risk:**<br>≥3 points (27 months) | **Low risk:**<br>0 points (14.6 years)<br>**Intermediate-1 risk**:<br>1–2 points (7.4 years)<br>**Intermediate-2 risk:**<br>3–4 points (4 years)<br>**High risk:**<br>5–6 points (2.3 years) | **Very low risk:**<br>0 points (not reached)<br>**Low risk:**<br>1–2 points (16.4 years)<br>**Intermediate-1 risk:**<br>3-4 points (7.7 years)<br>**High risk:**<br>5–8 points (4.1 years)<br>**Very high risk:**<br>≥9 points (1.8 years) | **Low risk:**<br>0 points (26.4 years)<br>**Intermediate-1 risk:**<br>1 point (8 years)<br>**Intermediate-2 risk:**<br>2 points (4.2 years)<br>**High risk:**<br>≥3 points (2 years) |

HMR - high molecular risk mutations include *ASXL1, SRSF2, EZH2, IDH1, IDH2* and, in addition, for GIPSS and MIPSS70-plus version 2.0, *U2AF1Q157*; VHR - very high risk karyotype include single/multiple abnormalities of -7, inv(3)/3q21, i(17q), 12p-/12p11.2 or 11q-/11q23, single/multiple autosomal trisomies other than +9 and +8; Unfavorable karyotype, any abnormal karyotype other than normal karyotype or sole abnormalities of 20q-, 13q-, +9, chr. 1 translocation/duplication, -Y, or sex chromosome abnormality other than –Y; Severe anemia, hemoglobin <8 g/dl in women and < 9 g/dl in men; Moderate anemia, hemoglobin 8–9.9 g/dl in women and 9–10.9 g/dl in men. Median survival is presented in months and years.

Table adapted from Gangat et al.[1]

Allogeneic hematopoietic stem cell transplant (AHSCT) is the only known treatment able to cure PMF, however, this procedure is associated with a very high risk of post-transplant complications and consequent death (*e.g*. graft vs. host disease).[3,8] Other treatment modalities as antimetabolites (hydroxyurea), *JAK2* inhibitors (ruxolitinib) and radiation therapy, mostly aim at symptomatic relief and spleen size reduction, as their use has not been shown to reverse fibrosis or induce remission in any degree.[3]

The natural progression of MNPs, can be divided into 3 different phases, defined according to the percentage of blasts in the BM or peripheral blood (PB): chronic phase (0–9%), accelerated phase (10–19%) and blast phase (≥20%).[9]

Leukemic progression (LP) is associated with an extremely unfavorable prognosis, occurring in approximately 20% of PMF patients.[3] ET and PV progress to accelerated phase and blast phase in a minority of cases, but LP is more frequent in PMF.[5] Current prognostic scoring systems are mostly focused on the overall survival (OS), therefore, since not all independent variables express a significant relation to LP, their capacity to accurately predict its risk is very limited.[9] Because of this, attempts are being made to identify predictors associated with disease progression to blast phase and AML.

Evidence suggests that increased levels of serum lactate dehydrogenase (LDH), at the time of initial diagnosis of PMF, have a significant correlation with poor prognosis and LP, in this sense, WHO and ICC have deliberately included increased levels of serum LDH as a minor criterion for the diagnosis of PMF (Table 1).[4] LDH is an enzyme of the anaerobic metabolic pathway, catalyzing the interconversion of lactate into pyruvate, and a quantitative marker of cell damage and turnover, widely available on the daily clinical practice.[10] However, no exact cut-off value was ever established for its usage in PMF prognostication.

Our study aims to evaluate a cohort of patients with PMF, diagnosed in a central hospital between 2010 and 2017 (with follow-up to 2022), characterize our demographic, clinical, analytical and genetic data, and investigate a possible correlation between increased serum LDH levels and LP as an early event, *i.e.,* within the first 5 years after initial diagnosis of PMF. Subsequently, we intend to establish the optimal cut-off value of serum LDH, for usage as an LP predictor in clinical practice.

## MATERIALS AND METHODS

*Research design*

The current study is a retrospective, observational and single-center cohort, developed in conformity with ethical and legal rights, namely with the *Helsinki Declaration*. Informed consent was not required from participants, as this study is retrospective, observational and an anonymization method was used.

*Study Population*

Our population consists of 53 patients diagnosed with PMF at a central hospital between 2010 and 2017, with follow-up to 2022, hence with a minimum follow-up time of 5 years. Diagnoses of PMF were established using WHO criteria, therefore, all patients included were submitted to a BM biopsy.[11] Finally, for LP diagnosis, a blast count of ≥20% in either the PB or BM was mandatory.[9]

The inclusion criteria utilized in our study were the following: established diagnosis of PMF according to WHO criteria, a minimum follow-up time of 5 years and the availability of serum LDH level at the time of initial diagnosis of PMF.

Regarding the exclusion criteria, a total of 107 patients with myelofibrosis were initially considered for our study, however, all cases of secondary myelofibrosis (17), either post-PV or ET, were excluded. The same applied to all patients with PMF first diagnosed after 2017 (36), as they did not comply with the 5-year follow-up inclusion criterion. Patients with LP that occurred more than 5 years after initial diagnosis of PMF (1) were also excluded from this study, in order to focus only on the “early“ events. Finally, serum LDH level at the time of initial diagnosis was available for all 53 patients, therefore, no further cases were excluded.

*Data collection*

All clinical data was collected retrospectively from patient’s files, starting at the time of initial diagnosis until their last follow-up or death, last updated on December 2022. In order to analyze the relevance of serum LDH in predicting LP at 5 years and further characterize our sample, variables that incorporate prognostic scores (IPSS; DIPSS; MIPSS70) were also collected, in addition to other predictors whose importance was seen as deserving of analysis. In this sense, the included variables, collected at the time of initial diagnosis, were: sex, age, constitutional symptoms, palpable splenomegaly, complete blood count (hemoglobin and anemia severity, mean corpuscular volume, leucocytes, platelets), transfusion dependency, ferritin levels, erythrocyte sedimentation rate (ESR), C-reactive Protein (CRP) levels, serum LDH levels, BM fibrosis grade, PB blast count, karyotype, driver and non-driver mutational status.

Anemia severity was registered as absent/mild (Hb ≥10g/dL), moderate (Hb 8–9.9g/dL) and severe (Hb <8g/dL).

Karyotype was divided into ”favorable“ (normal karyotype, 13q−, +9, 20q−, chromosome 1 translocation/duplication or sex chromosome abnormality including -Y), ”very high risk” (VHR) [−7, i(17q), inv(3)/ 3q21, 12p−/12p11.2, 11q−/11q23, other autosomal trisomies not including +8/ +9] and ”unfavorable” (any abnormal karyotype, other than normal karyotype or sole abnormalities of 20q-, 13q-, +9, chr. 1 translocation/duplication, -Y, or sex chromosome abnormality other than –Y).[1,12]

The included HMR (non-driver) mutational genes were: *ASXL1, EZH2, SRSF2, IDH1/2* and *U2AF1.*[12]

*Statistical analysis*

All statistical analyses were elaborated using *IBM's Statistical Package for Social Sciences 28.0* software.

Nominal variables are presented as frequency (absolute or relative), and continuous variables as median and range.

Two separate statistical methods were performed in order to assess the effect of increased serum LDH on the likelihood of LP in 5 years' time after initial diagnosis of PMF.

Binary outcome logistic regression statistics was employed to demonstrate a positive relation between serum LDH and LP within the first 5 years, in univariate and multivariate analyses. Variables that comprise DIPSS were included in the multivariate logistic regression model (age >65, constitutional symptoms, hemoglobin <10 g/dL, leucocytes >25 $x10^9$/L, peripheral blood blast count ≥1%), in addition to serum LDH level at the time of initial diagnosis of PMF.

Subsequently, a Receiver Operating Characteristics (ROC) analysis curve was created, based on serum LDH level at the time of initial diagnosis, along with calculation of the area under the curve (AUC), to further assess probability of LP at 5 years. By calculating the AUC, the model's performance can be classified as excellent (AUC between 0.9 and 1), good (0.8-0.9), fair (0.7-0.8), poor (0.6-0.7) or fail (<0.6).

In order to establish the optimal serum LDH level cut-off point, *i.e.,* the maximum potential effectiveness in terms of sensitivity and specificity to predict LP in 5 years-time, Youden Index (J) was used.

A *P* value of <0.05 was considered statistically significant.

## RESULTS

Our study included 53 patients with PMF [median age of 68 years (range 24-92), 62.3% males] followed at a central hospital between April 2010 and December 2022. Demographic, clinical, analytical and genetic data of all patients, at the time of initial diagnosis of PMF, is outlined in Table 3.

**Table 3.** Demographic, clinical, analytical and genetic data of all 53 patients, at the time of initial diagnosis of PMF

| **Variables** | **Total patients (n=53)** | **Leukemic progression within the first 5 years after diagnosis of PMF (n=8)** | **No leukemic progression within the first 5 years after diagnosis of PMF (n=45)** |
|---|---|---|---|
| Female – n (%) | 20 (37.7) | 2 (25) | 18 (40) |
| Male – n (%) | 33 (62.3) | 6 (75) | 27 (60) |
| Age at diagnosis, in years – median (range) | 68 (24-92) | 69 (55-83) | 68 (24-92) |
| Age >65 – n (%) | 33 (62.3) | 6 (75) | 27 (60) |
| Constitutional symptoms – n (%) | 21 (39.6) | 7 (87.5) | 14 (31.1) |
| Palpable splenomegaly – n (%) | 29 (54.7) | 7 (87.5) | 22 (48.9) |
| Hemoglobin, g/dL – median (range) | 12 (4.4-18.6) | 10.9 (8.7-15.9) | 12 (4.4-18.6) |
| Absent/mild anemia – n (%) | 39 (73.6) | 4 (50) | 35 (77.8) |
| Moderate anemia – n (%) | 11 (29.8) | 4 (50) | 7 (15.6) |
| Severe anemia – n (%) | 3 (5.7) | – | 3 (6.7) |
| Transfusion dependent – n (%) | 15 (28.3) | 4 (50) | 11 (24.4) |
| Mean corpuscular volume – median (range) | 86.5 (69.1-107.6) | 82 (72-91.5) | 87.1 (69.1-107.6) |
| Leukocyte count, $x10^9$/L – median (range) | 10.7 (1.30-66.9) | 10 (5.40-66.9) | 10.9 (1.30-21.6) |
| Leukocyte count >25 $x10^9$/L – n (%) | 1 (1.9) | 1 (12.5) | – |
| Platelet count, $x10^9$/L – median (range) | 454 (33-1950) | 300 (42-608) | 469 (33-1950) |
| Platelet count <100 $x10^9$/L – n (%) | 5 (9.4) | 1 (12.5) | 4 (8.9) |
| Ferritin, ng/mL – median (range) | 396.4 (7-3983) | 300.5 (47-894) | 396.4 (7-3983) |
| Erythrocyte sedimentation rate, mm/hr – median (range) | 20 (1-111) | 22 (2-76) | 19 (1-111) |
| C-reactive Protein, mg/dL – median (range) | 1.52 (0.07-8.30) | 1.54 (0.2-4.2) | 1.52 (0.07-8.30) |
| **Serum lactate dehydrogenase, U/L – median (range)** | **414 (124-992)** | **596 (397-992)** | **372 (124-875)** |
| **Serum lactate dehydrogenase, times the upper limit of normal – median (range)** | **1.67 (0.5-4)** | **2.4 (1.6-4)** | **1.5 (0.5-3.53)** |
| Bone marrow fibrosis grade ≥2 – n (%) | 31 (58.5) | 6 (75) | 25 (55.6) |
| Peripheral blood blast count % – median (range) | 0 (0-10) | 0 (0-1) | 0 (0-10) |
| Peripheral blood blast count ≥1% – n (%) | 7 (13.2) | 2 (25) | 5 (11.1) |

| Variables | Total patients (n=53) | Leukemic progression within the first 5 years after diagnosis of PMF (n=8) | No leukemic progression within the first 5 years after diagnosis of PMF (n=45) |
|---|---|---|---|
| Karyotype available,"N" evaluable = 15 – n (%) | 15 (28.3) | 3 (37.5) | 12 (26.7) |
| Favorable – n (%) | 10 (66.7) | 3 (100) | 7 (58.3) |
| Unfavorable – n (%) | 3 (20) | – | 3 (25) |
| Very high risk – n (%) | 2 (13.3) | – | 2 (16.7) |
| Driver mutational status available, "N" evaluable = 53 – n (%) | 53 (100) | 8 (100) | 45 (100) |
| *JAK2V617* – n (%) | 39 (73.6) | 7 (87.5) | 32 (71.1) |
| *MPL* – n (%) | 2 (3.8) | – | 2 (4.4) |
| *CALR* – n (%) | 8 (15.1) | 1 (12.5) | 7 (15.6) |
| Triple negative – n (%) | 4 (7.5) | – | 4 (8.9) |
| High molecular risk mutational status available,"N" evaluable = 18 – n (%) | 18 (34) | 2 (25) | 16 (35.6) |
| No high molecular risk mutations present – n (%) | 12 (66.7) | 2 (100) | 10 (62.5) |
| *ASXL1* – n (%) | 4 (22.2) | – | 4 (25) |
| *U2AF1* – n (%) | 2 (11.1) | – | 2 (12.5) |
| *EZH2* – n (%) | – | – | – |
| *SRSF2* – n (%) | – | – | – |
| *IDH1* – n (%) | – | – | – |
| *IDH2* – n (%) | – | – | – |
| Leukemic Progression within 5 years – n (%) | 8 (15.1) | | |

Among the 53 study participants, 62.5% had over 65 years of age, 39.6% presented with constitutional symptoms and 54.7% with palpable splenomegaly, at the time of initial PMF diagnosis.

Median hemoglobin was 12 g/dL, being that 73.6% of patients presented with normal Hb levels or mild anemia (Hb ≥10 g/dL), 29.8% with moderate anemia (Hb 8–9.9 g/dL) and 5.7% with severe anemia (Hb <8 g/dL). 28.3% of patients were transfusion dependent. Median leukocyte count was 10.7 $x10^9$/L, median platelet count was 454 $x10^9$/L, ESR had a median value of 20 mm/hr and CRP of 1.52 mg/dL. BM fibrosis grade ≥2 was present in 58.5% of patients and peripheral blood blast count ≥1% in 13.2%.

Among 15 evaluable patients, 66.7% presented with favorable karyotype, 20% with unfavorable karyotype and 13.3% had VHR karyotypes.

Regarding the driver mutational status, 73.6% had a *JAK2V617* gene mutation, 15.1% had a *CALR* mutation and 3.8% carried a *MPL* gene mutation, being that 7.5% had a triple negative status.

In 18 evaluable patients, HMR mutational status for the *ASXL1* gene mutation was 22.2%, 11.1% for *U2AF1*, being that 66.7% had no HMR mutations detected.

The median level of serum LDH was 414 U/L, being 1.67 times the adopted upper limit of normal (xULN), established at 248 U/L. The “LP within 5 years” and “no LP within 5 years” cohorts had a median serum LDH level of 596 U/L (2.4 xULN) and 372 U/L (1.5 xULN), respectively.

LP within 5 years since initial diagnosis of PMF occurred in 15.1% of patients.

In the univariate binary outcome logistic regression, the risk of LP within 5 years was associated with an increased serum LDH level at the time of initial diagnosis of PMF ($\chi2(1) = 8.177$, $p$=0.004). The model explained 25.0% (Nagelkerke $R^2$) of the variance in LP within 5 years and correctly classified 84.9% of cases. The likelihood of LP in 5 years’ time increased 0.6% by each increasing U/L of serum LDH, at the time of initial diagnosis of PMF [odds ratio (OR) 1.006, 95% confidence interval (CI) 1.001-1.010, $p$=0.010].

Multivariate analysis, only including DIPSS parameters (age >65, constitutional symptoms, hemoglobin <10 g/dL, leucocytes >25 x$10^9$/L, peripheral blood blast count ≥1%) in addition to serum LDH level, further confirmed that LDH has an independent contribution to predicting LP in 5 years’ time ($\chi2(7) = 22.169$, $p$=0.002). The model explained 59.8% (Nagelkerke $R^2$) of the variance in LP within 5 years and correctly classified 88.7% of cases. The likelihood of LP in 5 years’ time increased 0.7% by each increasing U/L of serum LDH, at the time of initial diagnosis of PMF (OR 1.007, 95% CI 1.001-1.012, $p$=0.025).

Serum LDH presented a significant correlation with age >65 years ($p$=0.045), constitutional symptoms ($p$=0.028), palpable splenomegaly ($p$=0,021), severe anemia ($p$=0,042) and decreased platelet count ($p$=0.030).

Figure 1 shows a ROC analysis curve, based on the level of serum LDH at the time of initial diagnosis of PMF and LP in 5 years' time, which presented an AUC of 0.807 or 80.7%. Utilizing Youden Index (J), a cut-off value for serum LDH was calculated at 522 U/L (2.105 xULN), with a sensitivity and specificity of 75% and 80%, respectively. The attained positive predictive value was 41.7%, with a negative predictive value of 94.38%.

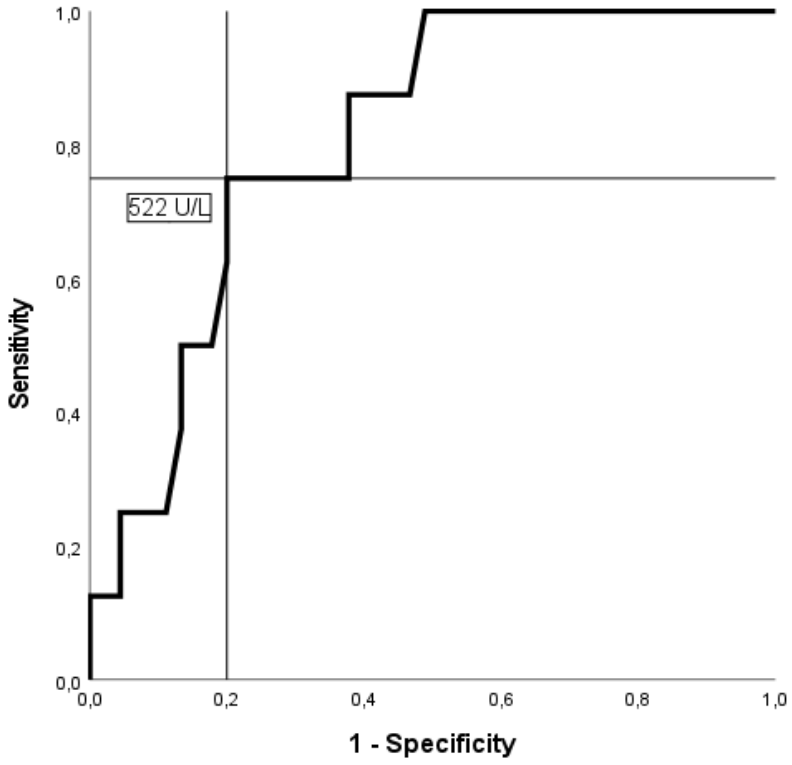


**Fig. 1.** ROC curve and cut-off value of serum LDH level at diagnosis of Primary Myelofibrosis, regarding leukemic progression in 5 years' time

## DISCUSSION/CONCLUSION

Conventional prognostic scoring systems are mostly focused on the OS of Myelofibrosis patients, although MIPSS70, MIPSS70-plus version 2.0 and GIPSS are able to also predict leukemic free survival (LFS).[6,13] Previous studies have considered as predictors of LP at the time of initial diagnosis of PMF: age >65 years [14], platelets <100 x$10^9$/L [15,16], CRP >7 mg/L [14], increased serum IL-8 and IL-2R [17], peripheral blood blast ≥3%[15] , bone marrow blasts >10%[18], unfavorable karyotype[18], triple negative driver mutational status [19] , *ASXL1, SRSF2* and *IDH1/2* gene mutations [20], among a few others and with different cut-off values for each variable.

In recent years, several attempts are being made to, not only identify new predictors associated with disease progression to blast phase and AML, but also to create new predictive models.

Serum LDH level is now known to be correlated with poor outcome in a variety of cancers, with extensive research about its prognostic usage being published.[21]

Our study, based on clinical data collected at a central hospital, demonstrates a positive correlation between increased serum LDH and LP within the first 5 years after initial diagnosis of PMF, and subsequently establishes the optimal cut-off point at 522 U/L (2.1 xULN), indicating that serum LDH levels ≥ 522 U/L independently predict LP in 5 years' time, with the highest achievable sensitivity and specificity.

Vallapureddy et al.[22], in a large, single-center retrospective study with 1306 PMF patients diagnosed between 1976 and 2017, elaborated an LP predictive model (in the first 5 years of disease and also over the course of the disease) based on clinical and genetic risk factors acquired at the time of initial diagnosis of PMF. They concluded that *IDH1, ASXL1, SRSF2* gene mutations, circulating blasts ≥3%, age >70 years and moderate/severe anemia were independently predictive of LP, and that their new model was superior to both MIPSS70-plus version 2.0 and GIPSS, in LP predictive accuracy. In regard to LP within the first 5 years; age >70 years, male sex, constitutional symptoms, moderate/severe anemia, circulating blasts ≥3%, *ASXL1*, *IDH1* and *SRSF2* gene mutations and VHR karyotype were deemed significant. However, although a substantial number of variables were tested, serum LDH was not among them.

In regard to other MPN, Tefferi et al.[23] , in a retrospective study of 216 PV patients about serum LDH prognostic relevance, concluded that serum LDH ≥1.5 xULN (established at 222 U/L) or ≥333 U/L was able to predict the risk of LP, also confirming serum LDH usefulness as an independent predictor of overall, leukemia-free and myelofibrosis-free survival in PV.

Mudireddy et al.[24], in a retrospective study of 183 consecutive ET patients, concluded that serum LDH is a leukocytosis-independent predictor of OS and that it might supersede the prognostic contribution of leukocytosis or thrombosis.

Later, Shah et al.[16] searched for a similar possibility in 311 PMF patients, in a single-center retrospective study about serum LDH prognostic utility, mostly regarding OS. They concluded that a marked increase of serum LDH (at time of referral) independently affects OS and LFS in PMF, and also that the prognostic contribution from serum LDH was independent of and possibly superior to leukocytosis. However, although a marked increase in serum LDH independently predicted LP (hazard ratio 3.1, 95% CI 1.2–7.6), no optimal cut-off point was calculated and statistical analysis was based on a pre-set cut-off value, given that ”marked elevation of serum LDH“ was established at ≥1000 U/L, over 4 xULN, established at 222 U/L.

In this sense, our study supports Shah et al.[16] by confirming that increased serum LDH is linked to LP, also supporting its independence from leukocytosis, since we did not find a significant correlation between serum LDH level and leucocyte count.

In a recent study regarding prognostic scoring in MF, Mosquera-Orgueira et al.[25] utilized machine learning to create a new scoring system, the Artificial Intelligence Prognostic Scoring System for Myelofibrosis (AIPSS-MF). Clinical data at initial diagnosis of 1386 MF patients was collected from a national database, being that 8 clinical variables retained significance and contributed to the AIPSS-MF ability to predict OS and LFS (age, sex, constitutional symptoms, hemoglobin level, leukocyte count, PB blast count, platelet count, and leukoerytroblastosis). Serum LDH level, at the time of initial diagnosis, was available in 757 patients. They concluded that serum LDH contribution to the model was neutral, as it did not enhance or diminish its predictability. Despite this, it should be noted that the exclusion of serum LDH from AIPSS-MF was based on the OS and not LFS, in addition to being determined from the total of patients (including both primary and secondary MF patients). Furthermore, the model focused on the OS and LFS, not the predictability of LP within 5 years' time, which differentiates its objective from our study. Testing serum LDH level as a continuous variable with no minimum cut-off point of significance (*e.g.* ≥500 U/L) may have also influenced its neutral contribution and subsequent exclusion from the AIPSS-MF model, since lower levels were considered for analysis. Lastly, adding another widely available clinical parameter (like serum LDH) to a clinical-based model, could contribute to its robustness without depriving it from its practical, non-genetical, approach.

The main limitation of our study derives from its limited number of participants, which emanates from being single-centered and only including patients diagnosed from 2010 onwards. Even tough smaller samples are associated with poorer statistical results, our findings seem to corroborate and enhance recent studies regarding serum LDH prognostic relevance in PMF. Despite this, its single-center format in addition to all data being collected in recent years, may reduce the risk of skewed data collection.

The chosen time period of 5 years for LP, since initial diagnosis of PMF, was based on its clinical usefulness regarding patient management. Besides, by studying LP only as an early (less than 5 years after diagnosis) and not a late event, we improve serum LDH ability to predict LP.

It is known that conditions like hemolysis, cancer, infection, liver and muscular disease, among others, can present with increased levels of serum LDH.[10,26] In this sense, another limiting factor of our study may originate from the absence of a method to diminish the impact of those comorbidities (unknown at the time of initial diagnosis and unrelated to PMF) in increasing the levels of serum LDH.

In closing, we concluded that in PMF patients, serum LDH levels ≥522 U/L at the time of initial diagnosis, independently predict LP in 5 years' time. Assuming this, whether serum LDH level should also be included in prognostic scoring for PMF, relies on further research to minimize confounding from the different methodology used in recent studies, to lessen the aforementioned limitations and to promote the usage of widely available clinical parameters, like serum LDH, extending PMF prognostic scoring to all centers.

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