Subcellular mislocalization of the transcription factor NF-E2 in erythroid cells discriminates pre-fibrotic primary myelofibrosis from essential thrombocytemia

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Running title: NF-E2 IHC discriminates prefibrotic PMF from ET

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KEY POINTS

The transcription factor NF-E2 is mislocalized in patients with primary myelofibrosis.

Immunohistochemical staining for nuclear factor erythroid-2 distinguishes essential thrombocythemia from primary myelofibrosis.

ABSTRACT

The WHO classification of myeloproliferative neoplasms (MPN) comprises several entities including essential thrombocythemia (ET), primary myelofibrosis (PMF) and MPN, unclassifiable (MPN,U). Differential diagnosis between ET and early, pre-fibrotic PMF can be challenging but is critical as clinical course and outcome vary considerably between these entities. We have previously shown that the transcription factor NF-E2 is aberrantly expressed in MPN patients. Here we demonstrate that NF-E2 is mis-localized in PMF cells and that aberrant NF-E2 localization discriminates statistically highly significantly between ET and PMF. A threshold of 20% nuclear NF-E2 staining was cross-validated by “.682+ bootstrapping”. Moreover, this cut-off correctly classifies diagnostic bone marrow biopsies of MPN,U patients specified upon follow-up as ET or PMF with 92% accuracy. Because inter-observer concordance between independent pathologists was high (Spearman’s rank correlation coefficient: 0.727), we propose that quantitative NF-E2 immunohistochemistry represents a diagnostic tool which can reliably support a differential diagnosis between ET and PMF.
INTRODUCTION

In 1951 Dr. William Dameshek first classified a group of clinically interrelated disorders, among them polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), naming them the myeloproliferative syndromes. The diagnostic criteria for these diseases, renamed myeloproliferative neoplasms (MPN), were last revised by the WHO in 2008. Despite the discovery of an activating point mutation in the JAK2 kinase (JAK2V617F) in the majority of MPN patients, the differential diagnosis between ET and PMF can remain challenging. Particular in the early stages, both the clinical presentation and the histopathological appearance of ET and PMF can be similar, a problem that has sparked lively discussion on distinguishing diagnostic criteria, including those of the WHO classification. In addition, the WHO classification has been criticized for relying on histopathology, which may be subject to a high degree of inter observer variability. However, because of the large difference in clinical course and outcome between ET and PMF, accurate classification and diagnosis of these entities is essential.

We have previously reported that expression of the transcription factor nuclear factor erythroid 2 (NF-E2) is aberrantly elevated in MPN patients. In ET and PMF, NF-E2 overexpression is independent of the presence or absence of the JAK2V617F mutation. Moreover, in a murine model, elevated NF-E2 levels cause an MPN phenotype.

Here we test the hypothesis that immunohistochemical staining for NF-E2 can be used to distinguish ET from PMF and that this distinction can aid in the classification of MPN patients.
Patients, Materials and Methods

Patients and bone marrow biopsies
A cohort of 163 bone marrow biopsies, which had been referred to and evaluated in the Institute of Pathology, Medical Center Freiburg between 2001 and 2010, was analyzed (Table 1). The first set consisted of 72 cases: 14 healthy controls (HC, obtained from patients with lymphoma biopsied for staging, that showed neither bone marrow infiltration nor complete blood count (CBC) abnormalities), 10 patients with reactive thrombocytosis (RT), 41 patients with essential thrombocythemia (ET), 39 patients with primary myelofibrosis (PMF; with the following fibrosis grades: MF-0 n=10, MF-1 n=19, MF-2 n=8, MF-3 n=2), and 33 patients with polycythemia vera (PV). The study was approved by the local internal review board (Albert-Ludwigs University, Freiburg, Germany). The study was conducted in accordance with the Declaration of Helsinki.

The second set consisted of 26 patients who presented with thrombocythemia. Of these 19 patients were initially diagnosed as MPN, unclassifiable (MPN,U). The WHO defines these as cases that show definite clinical, laboratory and morphological features of a myeloproliferative neoplasm, but that fail to meet the criteria for any of the specific MPN entities. By follow-up biopsy, one to nine years later, and by clinical course, these 19 cases were subsequently diagnosed as either ET (n=10; MPN,U-ET) or PMF (n=9; MPN,U-PMF).

In addition, the second set contained 7 patients (here called ET-PMF) initially interpreted by us as ET, who were, however, found to satisfy the criteria for PMF, including characteristic histological atypia, in the follow-up biopsy one to eight years later. These are nonetheless not examples of ET that developed fibrosis, so called post ET-MF by WHO definition, as this WHO definition of post-ET-MF requires a degree of fibrosis grade 2, which these patients did not show. In addition, these patients transformed to PMF with a median of 1 year of the initial diagnosis, six of the seven patients transformed within 2 years of diagnosis. Post ET-MF, when it occurs, takes much more time to develop, patients transforming with a median of 8-9 years after diagnosis (range 3.6 – 20.2) .

All cases were diagnosed according to the WHO criteria . In order to achieve pathologic diagnoses by consensus of multiple observers, three hematopathologists (K.A., A.M.M. and M.W.), each blinded to both the initial diagnosis, as
well as to the diagnoses of his or her colleagues, re-reviewed all biopsies, which were stained for CAE (chloracetate-esterase), Giemsa, H & E (hematoxylin and eosin) as well as reticulin (Gomorri stain). Only those patients were chosen for inclusion into this study, for which all three pathologists independently arrived at the same diagnosis according to WHO criteria, and for which this diagnosis was in agreement with the initial diagnosis.

Importantly, this panel of pathologists arrived at the same diagnosis and matched the initial diagnosis in 113/125 (90.4%) of cases. Only 12 out of an initially available pool of 125 biopsies (9.6%) had to be excluded because of inter-pathologist disagreement. In addition to the histologies, the pathologists had the following clinical information: CBC for all patients, LDH for the majority of patients, splenomegaly for a subset of patients, mainly those with suspected PMF, JAK2V617F for all suspected PV cases as well as the majority of suspected ET and PMF cases.

The biopsies used for NF-E2 staining were obtained at initial diagnosis and were therapy naïve.

**NF-E2 and CD71 Immunohistochemistry**

Bone marrow biopsies were either fixed in 4% buffered formalin (FA) or in “Calcium-Glutardialdehyde-Formaldehyd, CGF” (0.1 mol/l calcium acetate, 1.1 vol % formaldehyde and 0.5 vol % glutardialdehyde), as described. Following fixation, all biopsies were subjected to decalcification in a mixture of 10 % ethylenediaminetetraacetic acid disodium salt (EDTA, Serva, Cat. No. 11280.02) and 3.3 % tris-(hydroxymethyl) aminomethane (THAM, AppliChem, Cat. No. A1086,1000) in dd H2O at pH: 7.0 – 7.2 over-night or for two days at room temperature, as described, and embedded in paraffin. Decalcification for either one or two days yielded identical staining results (data not shown).

Serial 3µm sections were de-paraffinized in xylene and graded alcohols, followed by specific antigen retrieval in “Target Retrieval Solution, pH9” in a steamer (Dako, Glostrup Denmark, Cat. No. S2367; 20 minutes for CGF fixed biopsies and 4-6 minutes for FA fixed biopsies, depending on the NF-E2 antibody lot). After incubation with one of two primary antibodies against NF-E2 for one hour at room temperature (NF-E2, polyclonal rabbit, Cat. No. HPA001914, Sigma Aldrich, diluted 1:50 for CGF-fixed biopsies and 1:200 for formalin fixed biopsies or anti-NF-E2, rabbit polyclonal 1089, diluted 1:100, raised against amino acids 133 – 147 of the NF-E2 protein21).
Staining was detected with the “Dako Real Detection System” (Dako, Cat. No. K5005, Sigma antibody) or the “Dako EnVision FLEX Visualization System” (Dako, Cat. No. K8000, antibody 1089). The sections were counterstained with hematoxylin (Dako, Cat. No. K8008) and mounted. To exclude unspecific staining, negative controls were prepared by replacing NF-E2 with an immunoglobulin isotype control (ChromPure Rabbit IgG, Jackson ImmunoResearch, Cat. No. 011-000-003).

For double staining with CD71, NF-E2 staining and detection was followed by a peroxidase block. Subsequently, an anti-CD71 antibody was applied for 20 minutes at room temperature (DCS, mouse monoclonal, clone H68.4; ready to use). Staining was detected with the “Dako EnVision FLEX Visualization System” (Dako, Cat. No. K8000).

NF-E2-specific staining was evaluated in 300 erythroid cells, 100 in each of three random high power fields (600 x magnification) per case. Every biopsy was evaluated independently by two pathologists, both blinded to the diagnosis. The percentage (mean +/- standard deviation) of nuclear positive, cytoplasmic positive, and negative erythroid cells as a proportion of all nucleated erythropoietic precursor cells was calculated for each biopsy. Cells that were both nuclear and cytoplasmic positive were regarded as nuclear positive.

**Statistical analysis**

Statistical analyses were performed after the data of both observers had been averaged. A two-tailed Wilcoxon test was used to determine whether a significant (p< 0.05) difference existed between two groups. When comparing more than two groups, a Kruskal Wallis One Way ANOVA on Ranks was used. A Spearman’s Rank Order correlation was run to determine the concordance between the two pathologists’ nuclear NF-E2 quantification.

The SPSS Software 18.0.2 (IBM Corporation, Somers, NY, USA) was used for analysis.

**Threshold Calculation and Cross Validation by .632+ Bootstrapping**

For threshold calculation, a classification was performed after rank transformation of the measurements using a Naïve Bayes classifier as shown in Fig. 3. A threshold of 20.3% was calculated to optimally discriminate between MPN,U-ET and MPN,U-PMF patients.
The classification error rate was derived by cross-validation using bootstrapping, a method that repeatedly divides the data into training- and test sets. For this analysis, 10,000 bootstrap data sets were drawn, of the same sample size as the experimental data set (10 MPN,U-ET and 9 MPN,U-PMF). The bootstrap sets are randomly selected from the experimental data set with replacement. For each of the 10,000 bootstrap data sets, the rank transformation was applied, the classification threshold calculated, and subsequently evaluated how often the classifier correctly predicts out-of-sample. For the 10,000 realizations, the average out-of-sample classification error was 9.7%. Because this number is known to overestimate the classification error, the so-called .632+ estimator 22 was applied for adjustment, such that the out-of-sample error is reasonably weighted with the in-sample classification error (5.3%). In our analysis, a weight of 0.66 was derived for the .632+ estimator yielding an expected classification error of 8.2% for validation measurements.
Results

Subcellular localization of NF-E2 during erythroid maturation

The transcription factor NF-E2 is known to be expressed in several hematopoietic lineages. However, its subcellular localization during distinct stages of erythroid differentiation is unknown. We therefore performed immunohistochemistry of healthy bone marrow biopsies and scored nuclear or cytoplasmic NF-E2 localization in various stages of erythroid maturation. All 14 biopsies were scored independently by two pathologists.

In healthy bone marrow, megakaryocytes were predominantly NF-E2 positive either in the cytoplasm, in the nucleus or in both compartments. In addition, cells of the myeloid lineage were weakly positive for NF-E2. Again both nuclear and cytoplasmatic staining was observed. Lymphocytes did not stain for NF-E2.

In early erythropoietic cells NF-E2 showed nuclear staining (Fig. 1A and B, arrows), whereas, unexpectedly, in later nucleated erythroid stages it was found almost exclusively in the cytoplasm (Figure 1A and B, filled arrowheads). Surprisingly for a transcription factor, on average only 10.9% (standard deviation, SD: +/- 3.8) of all erythropoietic cells, mainly the early erythroblasts, showed a nuclear NF-E2 positivity (Fig. 1C), whereas the vast majority, on average 85.7% (+/- 2.2%), stained cytoplasmatically (Fig. 1C). Very few erythroid cells (an average of 3.4% +/- 2.5%) were completely negative for NF-E2 (Figure 1A-C). This is the first description of strong cytoplasmic NF-E2 expression in more mature erythropoietic cells and raises the possibility that the NF-E2 protein may fulfill additional, previously unrecognized functions in the cytoplasm.

NF-E2 is mis-localized in MPN biopsies and shows increased nuclear staining

Because of the documented NF-E2 overexpression in MPN patients we hypothesized that NF-E2 staining or subcellular localization may differ between MPN patients and healthy controls. We therefore assembled a cohort of MPN bone marrow biopsies, diagnosed according to the WHO criteria, consisting of n=33 PV, n=41 ET and n=39 PMF cases. In addition to fulfilling the WHO classification criteria, most cases had obtained follow up biopsies at least one year after the initial diagnosis. At this time, the MPN entity was confirmed both by pathological analysis and by clinical course. The biopsies obtained at the time of diagnosis were stained
for NF-E2 and again scored for subcellular localization in erythroid cells. The two independently scoring pathologists were blinded to the diagnoses.

Like healthy controls, ET cases showed a low proportion of nuclear positivity for NF-E2 (16.3% +/- 4.1% of all erythroid cells; Fig. 1D and F). Patients with reactive thrombocytosis, which serve as an important control, likewise showed low numbers of NF-E2 nuclear positive erythroid cells (15.1 +/- 3.4% 1F). In contrast, PV cases showed a slightly higher proportion of nuclear positivity in erythroid cells (23.9% +/- 10%) and this difference reached statistical significance (Fig. 1F; p<0.0001 vs. HC and ET, respectively). Most striking is the stark and statistically highly significant increase in nuclear NF-E2 staining observed in PMF patients (33.7% +/- 10.7%: p<0.0001 vs PV, ET and HC respectively; Fig. 1E and F). This marked increase in the proportion of NF-E2 nuclear positive erythroid cells was apparent even in patients with grade 0 or 1 fibrosis, suggesting that increased nuclear staining of the transcription factor is inherent to disease development, rather than being a feature of disease progression.

In order to verify that the cells scored for NF-E2 staining were indeed erythroblasts, we conducted double stainings with NF-E2 and CD71, a marker of early erythroid cells. These data were quantitated and are shown in Suppl. Fig. 2. They reveal that indeed, firstly, more than 95% of all cells that stain nuclear for NF-E2 in the bone marrow are CD71-positive erythroblasts (Suppl. Fig. 2C), and, secondly, that, counting only CD71 positive cells, hence cells that have been identified as erythroid due to a surface marker, PMF patients show a statistically highly significant increase in nuclear NF-E2 staining (Suppl. Fig. 2D).

We wished to confirm these differences in NF-E2 staining with a second, alternative antibody, generated in our lab and raised against a different NF-E2 peptide than the antibody initially used. Cytoplasmic and nuclear NF-E2 staining in erythroid cells was again quantitated in ET and PMF patients. As with the first antibody used, a statistically highly significant difference between ET and PMF patients was observed (Suppl. Fig.1). This second antibody therefore confirms both the cytoplasmic staining of NF-E2 in mature erythroid cells and the mislocalization of NF-E2 in PMF patients.

Because early erythropoiesis shows nuclear NF-E2 staining, while more mature erythroid cells display cytoplasmic NF-E2 (Fig. 1A-C), the increase in NF-E2 nuclear staining observed in PMF could simply result from the presence of increased
numbers of early erythroblasts in PMF bone marrow. We therefore quantified the percentage of early and late erythropoietic cells in ET and PMF patients (Fig. 1G). There was no difference in the percentage of early and late erythropoietic cells between ET and PMF. Rather, a significantly higher percentage of early erythroblasts showed nuclear NF-E2 staining in PMF compared to ET, demonstrating that the NF-E2 protein is indeed mis-localized in PMF (Fig. 1H). Aberrant localization could occur either by abnormal retention in the nucleus, by insufficient cytoplasmic export, or by increased re-import into this organelle.

Because the percentage of nuclear NF-E2 positivity in erythroid cells is highly significantly different between PMF and ET biopsies (p<0.0001, Fig. 1F), we proposed that this staining could be used to discriminate between the early, prefibrotic stage of PMF, and ET, entities which are often challenging to differentiate diagnostically.

**Quantitative NF-E2 immunohistochemistry discriminates between early, prefibrotic PMF and ET**

In order to test the hypothesis that NF-E2 staining can discriminate between early, pre-fibrotic PMF and ET, we analyzed a second MPN cohort. This cohort again consisted entirely of cases which were diagnosed according to the WHO criteria and had both follow-up biopsies and clinical data available for all patients. The WHO classification recognizes an entity termed “MPN-Unclassifiable (MPN,U)” for MPN cases who do not clearly fulfill the diagnostic criteria for either PV, ET or PMF. Upon follow-up, these patients, if they now fulfill the WHO criteria for another MPN entity, may be reclassified. Our second cohort consisted of n=19 MPN,U patients, 10 of which were re-classified as ET upon follow up (MPN,U-ET, Fig. 2A) and 9 re-classified as PMF upon follow up (MPN,U-PMF, Fig. 2B). In addition, we included 7 patients whose initial diagnosis of ET was revised to PMF upon follow-up (ET-PMF, Fig 2C).

The proportion of nuclear NF-E2 positive erythroid cells in the initial biopsies of MPN,U cases later diagnosed as ET was as low as that observed in ET patients (14.8% +/- 4.3%; Fig. 2D). In contrast, NF-E2 nuclear staining in initial biopsies of MPN,U cases later reclassified as PMF (27.6% +/- 5.4%) was statistically highly significantly elevated compared to either ET or to MPN,U-ET cases (both p<0.0001; Fig. 2D) and similar to that of PMF cases (Fig. 2D). Likewise, biopsies of patients
initially diagnosed as ET, who were later reclassified as PMF cases revealed a high proportion of NF-E2 nuclear positive erythroid cells in their initial biopsies (27.6% +/- 6.7%), comparable to that of PMF cases (Fig. 2D). ET-PMF cases differ highly significantly from both ET (p<0.0001) and from MPN,U-ET cases (p<0.01) Fig. 2D).

We therefore propose that NF-E2 immunohistochemistry allows a discrimination of MPN patients with ET from those with early, prefibrotic PMF, which is especially important for those patients whose clinically presentation and bone marrow morphology does not allow this differential diagnosis. The threshold for NF-E2 nuclear positivity was calculated to be 20%, samples showing more nuclear NF-E2 positive erythropoiesis can be classified as PMF, while those showing less are classified as ET (Fig. 3A). In order to validate this threshold, we performed a “.682+ bootstrapping” cross-validation. The 10,000 bootstrap data sets generated predict a correct distinction between ET and pre-fibrotic PMF in 92% of cases, hence an error rate of 8%, using 20% nuclear NF-E2 as a cut off (Fig. 3B).

Morphological analysis has been criticized, especially in the MPN field, for being difficult to apply in daily practice as it may be subject to considerable inter-observer variability. We therefore calculated the inter observer variability between the two pathologists, who scored the biopsies blinded both to the diagnosis as well as to the results of the other researcher. The Spearman's rank correlation coefficient of 0.727 demonstrates an extremely high inter-observer consistency for all 163 cases (p<0.001).
Discussion

Despite the rising number of molecular aberrations detected in MPN patients, the distinction between the three related entities, especially between the early, pre-fibrotic phase of PMF and ET remains difficult and relies mainly on histology and clinical diagnosis. The reason is that none of the mutations detected in MPN patients are exclusive to any one entity. In addition, many of the mutations are found in a small subset of patients (5 – 10%) and the costs of searching for 10 possible mutations in any one patient are prohibitive given the current technology. Therefore, in patients with isolated thrombocytosis, histology remains one of the most important tools for distinguishing ET from pre-fibrotic PMF.

However, it has been criticized that histology alone is insufficient in its discriminatory power and inter-observer variability remains a concern. Additional tools for distinction of ET from pre-fibrotic PMF would clearly be beneficial not only for diagnosis, but also for the interpretation of clinical trial results. For example, the PT1 trial, which investigated the use of hydroxyurea and anagrelide in ET has been criticized for including pre-fibrotic PMF patients in its cohort.

Here we describe a simple, highly reproducible immunohistochemical stain, which demonstrated a low inter-observer variability, and a high degree of accuracy. Interestingly, the proportion of nuclear NF-E2 staining in PMF patients is already significantly elevated at diagnosis and remains stable during follow-up (Suppl. Fig. 3). Our data indicate that analysis of the proportion of nuclear NF-E2 positive erythroid cells represents a viable diagnostic tool which can add a highly reliable support to reaching a differential diagnosis between ET and PMF.

In summary, quantitative NF-E2 immunohistochemistry of bone marrow biopsies of MPN patients presenting with thrombocytosis can help to distinguish ET from early, prefibrotic PMF, with important consequences for both therapeutic decisions and prognostic implications.
Acknowledgements

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Author Contributions

KA designed experiments, conducted the experiments analyzed the data and wrote the manuscript. AVF, AMM, JM, CK, JT and DH analyzed data. MW analyzed data and critically reviewed the manuscript. HLP designed experiments, analyzed the data and wrote the manuscript.

Conflict of Interest

None of the authors have any conflict of interest to declare.
REFERENCES


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**Table 1: Clinical data at the time of the initial biopsies.** WBC: white blood cell count x 10⁶ per µl; PLT: platelet count x 10⁶ per µl; HB: hemoglobin in g/dl; LDH: lactate dehydrogenase in U/l; ns: not specified; * data was not available for the remainder of the patients.
Figures and Figure Legends

Figure 1: Immunohistochemistry of NF-E2 in healthy controls, reactive thrombocytosis, ET, PV and PMF patients. (A and B, healthy controls; D, Essential Thrombocythemia; E, Primary Myelofibrosis) Bone marrow biopsies were stained with an antibody against NF-E2 and counterstained with hematoxylin (1000x original magnification, bar indicates 20µm). Arrows point to erythropoietic cells with nuclear NF-E2 staining, filled arrow heads indicate cytoplasmic NF-E2 staining, whereas open arrowheads mark cells negative for NF-E2 staining. (C, F-H) One hundred erythropoietic cells in each of three high power fields per bone marrow biopsy (n=300 erythroid cells in total) were evaluated for NF-E2 subcellular localization and/or differentiation stage. (C) Quantitative analysis of NF-E2
**subcellular localization in healthy controls.** Shown is the percentage of nuclear or cytoplasmic NF-E2 positive erythroid cells, respectively, as well as NF-E2 negative cells as a proportion of all erythroid precursors. (F) Quantitative analysis of nuclear NF-E2 positivity in erythroid cells in healthy controls, reactive thrombocytosis (RT) and MPN patients. Shown is the percentage of nuclear NF-E2 positive erythroid cells as a proportion of all erythroid precursors. *p<0.05; ***p<0.001, ****p<0.0001 by two-tailed Wilcoxon-test.

(G) Proportion of early and late erythroblasts of all erythroid cells in ET and PMF. Shown is the percentage of early and late erythroblasts as a proportion of all erythroid cells in ET and PMF. An early erythroblast was defined on a CAE stain as a CAE negative erythroid cell with a small cytoplasm, a large nucleus (1.5 – 2.5 fold of the diameter of an erythrocyte), and one or two prominent nucleoli. A late erythroblast was defined as a CAE negative cell, with abundant cytoplasm, frequently polygonal in shape, and with a round nucleus with dense chromatin. (H) Proportion of NF-E2 nuclear or cytoplasmic positive early erythroblasts in ET and PMF. Shown is the percentage of nuclear or cytoplasmic NF-E2 positive cells, as indicated, in early erythroid precursors. ***p<0.001 by two-tailed Wilcoxon-test.
Figure 2: Immunohistochemistry of NF-E2 in MPN, Unclassifyable, ET, and PMF patients. Bone marrow biopsies were stained with an antibody against NF-E2 and counterstained with hematoxylin (1000x original magnification, bar indicates 20µm). Arrows point to erythropoietic cells with nuclear NF-E2 staining, filled arrow heads indicate cytoplasmic NF-E2 staining.

(A) MPN, Unclassifyable later re-classified as ET (B) MPN, Unclassifyable later re-classified as PMF. (C) ET later re-classified as PMF (D) Quantitative analysis of NF-E2 immunohistochemistry in MPN, U patients. One hundred erythropoietic cells in each of three high power fields per bone marrow biopsy were evaluated (n=300 erythroid cells in total). Shown is the percentage of nuclear NF-E2 positive erythroid cells as a proportion of all erythroid precursors. Data for ET and PMF as in 1F; **p<0.01, ***p<0.001, ****p<0.0001 by Wilcoxon-test.
Figure 3: Rank Transformation and Threshold Calculation

Rank transformations constitute an efficient strategy to perform robust analyses with respect to distributional assumptions.28

(A) Posterior probability densities for MPN, U-ET and MPN, U-MPF patients fitted to the measurements after rank transformation. A rank transformation of the data was applied to perform the classification analysis. The posterior probability densities are plotted for both groups, MPN,U-ET and MPN,U-PMF patients. The threshold of 10.48 for the ranks is obtained by determining the rank with equal posterior probabilities.

(B) Transformation: The threshold value for the ranks (10.48) was translated to the measurement scale. Ranks are plotted in vertical direction, the horizontal axis denotes the measurements as % nuclear positive cells. The threshold 10.48 for ranks (vertical axis) corresponds to 20.26% nuclear positive cells (horizontal axis).
Subcellular mislocalization of the transcription factor NF-E2 in erythroid cells discriminates pre-fibrotic primary myelofibrosis from essential thrombocythemia

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